

FIG. 1A

pET22b(+) forward primer:

5'-CGGGATCCT TCT GTT GAT CAC GGC TTC-3' (SEQ ID NO:3)

pET22b(+) reverse primer:

5'-CCCAAGCTT TGT TCT TCT CAT ACA GAC-3' (SEQ ID NO:4)

pPICZαA forward primer:

5'-TTCGGAATTC TCT GTT GAT CAC GGC TTC-3' (SEQ ID NO:15)

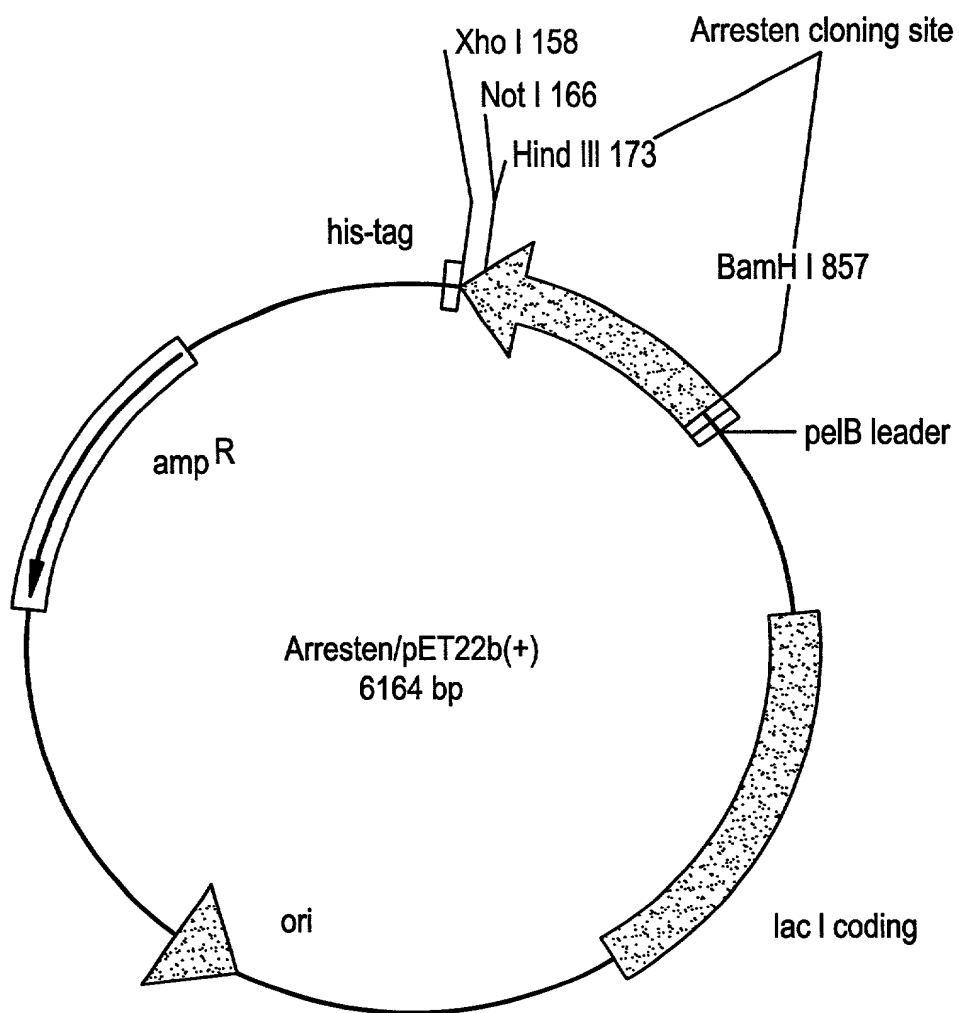
pPICZαA reverse primer:

5'-TGCTCTAGAGG TGT TCT TCT CAT ACA GAC TTG GCA-3' (SEQ ID NO:16)

| | | | | | | | | | | | | | | |
|--------------------------------|------------|------------|------------|-----|---------------|-----|-----|-----|-----|-----|------------|------------|------------|------------|
| 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | | | | | | |
| <u>tct gtt gat cac ggc ttc</u> | ctt | gtg | acc | agg | cat | agt | caa | aca | ata | | | | | |
| 50 | 55 | 60 | 65 | 70 | 75 | 80 | 85 | 90 | | | | | | |
| gat | gac | cca | cag | tgt | cct | tct | ggg | acc | aaa | att | ctt | tac | cac | ggg |
| 95 | 100 | 105 | 110 | 115 | 120 | 125 | 130 | 135 | | | | | | |
| tac | tct | ttg | ctc | tac | gtg | caa | ggc | aat | gaa | cgg | gcc | cat | gga | cag |
| 140 | 145 | 150 | 155 | 160 | 165 | 170 | 175 | 180 | | | | | | |
| gac | ttg | ggc | acg | gcc | ggc | agc | tgc | ctg | cgc | aag | ttc | agc | aca | atg |
| 185 | 190 | 195 | 200 | 205 | 210 | 215 | 220 | 225 | | | | | | |
| ccc | ttc | ctg | ttc | tgc | aat | att | aac | aac | gtg | tgc | aac | ttt | gca | tca |
| 230 | 235 | 240 | 245 | 250 | 255 | 260 | 265 | 270 | | | | | | |
| cga | aat | gac | tac | tgc | tac | tgg | ctg | tcc | acc | cct | gag | ccc | atg | ccc |
| 275 | 280 | 285 | 290 | 295 | 300 | 305 | 310 | 315 | | | | | | |
| atg | tca | atg | gca | ccc | atc | acg | ggg | gaa | aac | ata | aga | cca | ttt | att |
| 320 | 325 | 330 | 335 | 340 | 345 | 350 | 355 | 360 | | | | | | |
| agt | agg | tgt | gct | gtg | tgt | gag | gcg | cct | gcc | atg | gtg | atg | gcc | gtg |
| 365 | 370 | 375 | 380 | 385 | 390 | 395 | 400 | 405 | | | | | | |
| cac | agc | cag | acc | att | cag | atc | cca | ccg | tgc | ccc | agc | ggg | tgg | tcc |
| 410 | 415 | 420 | 425 | 430 | 435 | 440 | 445 | 450 | | | | | | |
| tgc | ctg | tgg | atc | ggc | tac | tct | ttt | gtg | atg | cac | acc | agc | gct | ggg |
| 455 | 460 | 465 | 470 | 475 | 480 | 485 | 490 | 495 | | | | | | |
| gca | gaa | ggc | tct | ggc | caa | gcc | ctg | gcg | tcc | ccc | ggc | tcc | tgc | ctg |
| 500 | 505 | 510 | 515 | 520 | 525 | 530 | 535 | 540 | | | | | | |
| gag | gag | ttt | aga | agt | gcg | cca | ttc | atc | gag | tgt | cac | ggc | cgt | ggg |
| 545 | 550 | 555 | 560 | 565 | 570 | 575 | 580 | 585 | | | | | | |
| acc | tgc | aat | tac | tac | gca | aac | gct | tac | agc | ttt | tgg | ctc | gcc | acc |
| 590 | 595 | 600 | 605 | 610 | 615 | 620 | 625 | 630 | | | | | | |
| ata | gag | agg | agc | gag | atg | ttc | aag | aag | cct | acg | ccg | tcc | acc | ttg |
| 635 | 640 | 645 | 650 | 655 | 660 | 665 | 670 | 675 | | | | | | |
| aag | gca | ggg | gag | ctg | cgc | acg | cac | gtc | agc | cgc | <u>tgc</u> | <u>caa</u> | <u>gtc</u> | <u>tgt</u> |
| 680 | 685 | 690 | | | | | | | | | | | | |
| <u>atg</u> | <u>aga</u> | <u>aga</u> | <u>aca</u> | taa | (SEQ ID NO:1) | | | | | | | | | |

| Year | 1990 | 1991 | 1992 | 1993 | 1994 | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 | 2024 | 2025 | 2026 | 2027 | 2028 | 2029 | 2030 | 2031 | 2032 | 2033 | 2034 | 2035 | 2036 | 2037 | 2038 | 2039 | 2040 | 2041 | 2042 | 2043 | 2044 | 2045 | 2046 | 2047 | 2048 | 2049 | 2050 | 2051 | 2052 | 2053 | 2054 | 2055 | 2056 | 2057 | 2058 | 2059 | 2060 | 2061 | 2062 | 2063 | 2064 | 2065 | 2066 | 2067 | 2068 | 2069 | 2070 | 2071 | 2072 | 2073 | 2074 | 2075 | 2076 | 2077 | 2078 | 2079 | 2080 | 2081 | 2082 | 2083 | 2084 | 2085 | 2086 | 2087 | 2088 | 2089 | 2090 | 2091 | 2092 | 2093 | 2094 | 2095 | 2096 | 2097 | 2098 | 2099 | 2100 |
|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 1990 | 1991 | 1992 | 1993 | 1994 | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 | 2024 | 2025 | 2026 | 2027 | 2028 | 2029 | 2030 | 2031 | 2032 | 2033 | 2034 | 2035 | 2036 | 2037 | 2038 | 2039 | 2040 | 2041 | 2042 | 2043 | 2044 | 2045 | 2046 | 2047 | 2048 | 2049 | 2050 | 2051 | 2052 | 2053 | 2054 | 2055 | 2056 | 2057 | 2058 | 2059 | 2060 | 2061 | 2062 | 2063 | 2064 | 2065 | 2066 | 2067 | 2068 | 2069 | 2070 | 2071 | 2072 | 2073 | 2074 | 2075 | 2076 | 2077 | 2078 | 2079 | 2080 | 2081 | 2082 | 2083 | 2084 | 2085 | 2086 | 2087 | 2088 | 2089 | 2090 | 2091 | 2092 | 2093 | 2094 | 2095 | 2096 | 2097 | 2098 | 2099 | 2100 | |

FIG. 2



Forward primer: 5'-cgggatccttctgttgatcacggcttc-3'

Reverse primer: 5'-cccaagcttggttcttcatacagac-3'

FIG. 3A

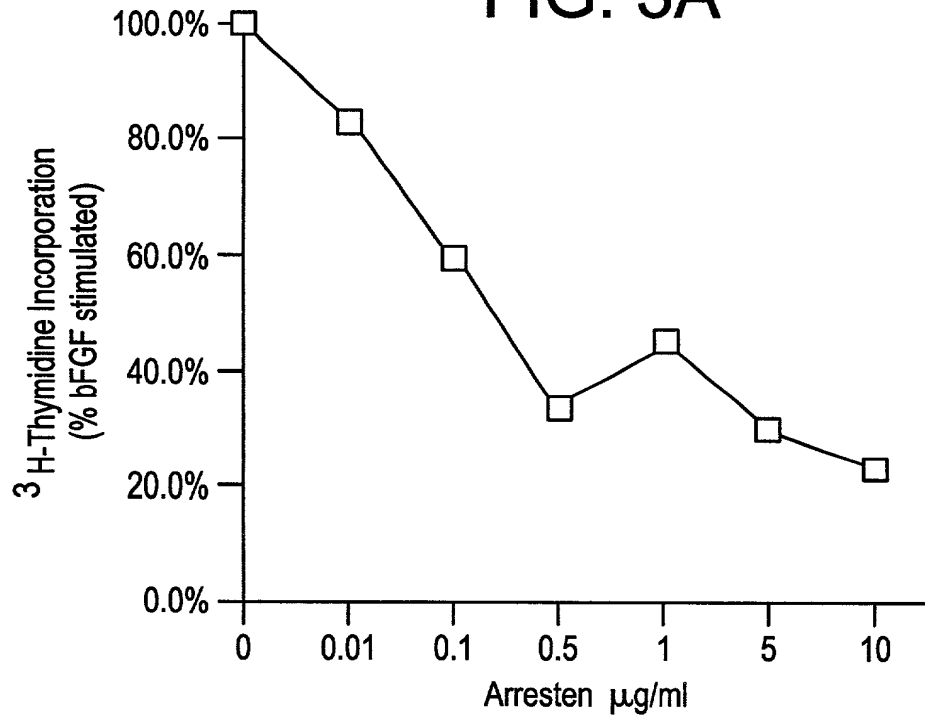


FIG. 3B

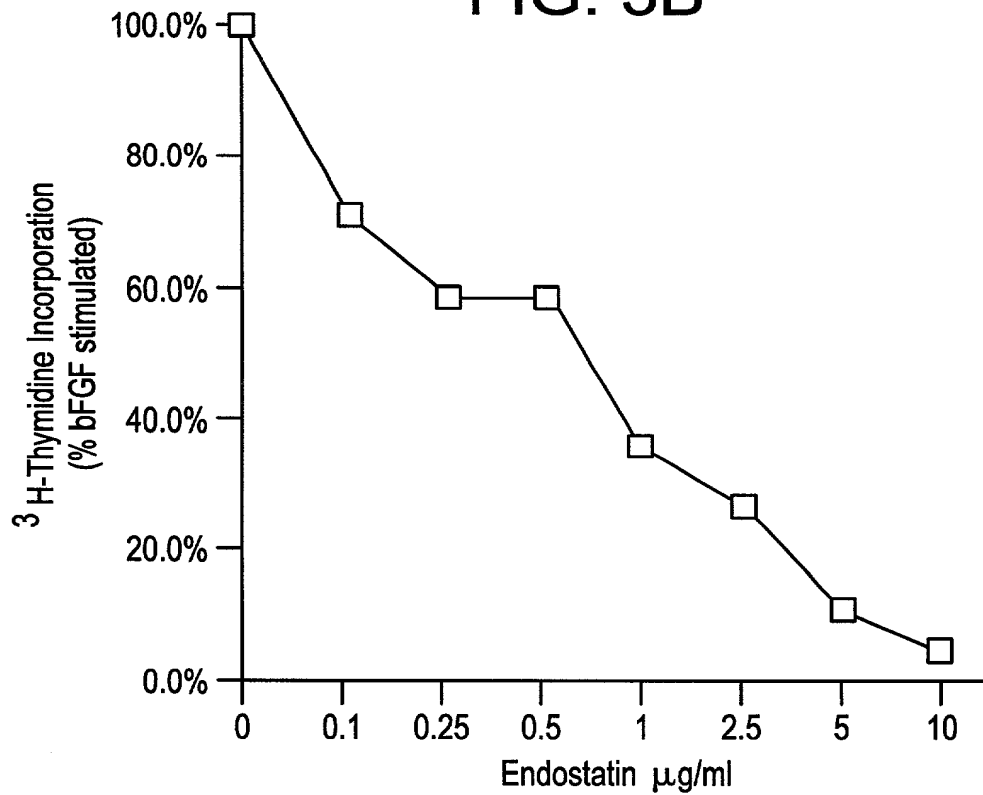


FIG. 4A

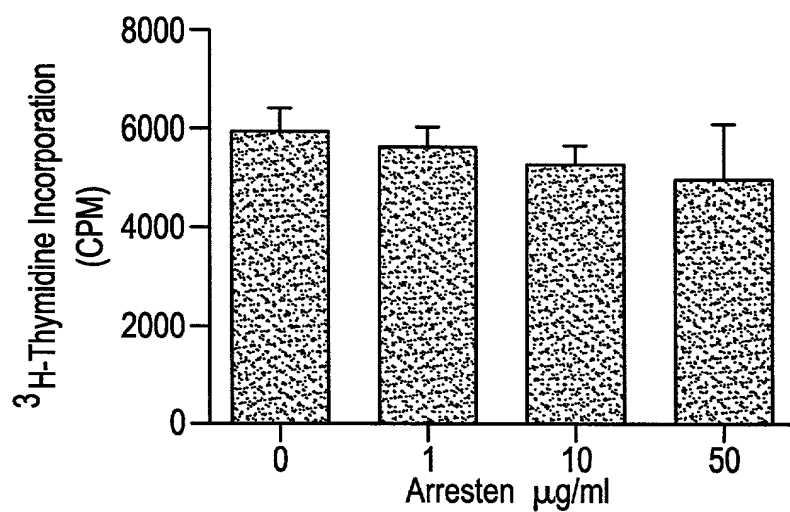


FIG. 4B

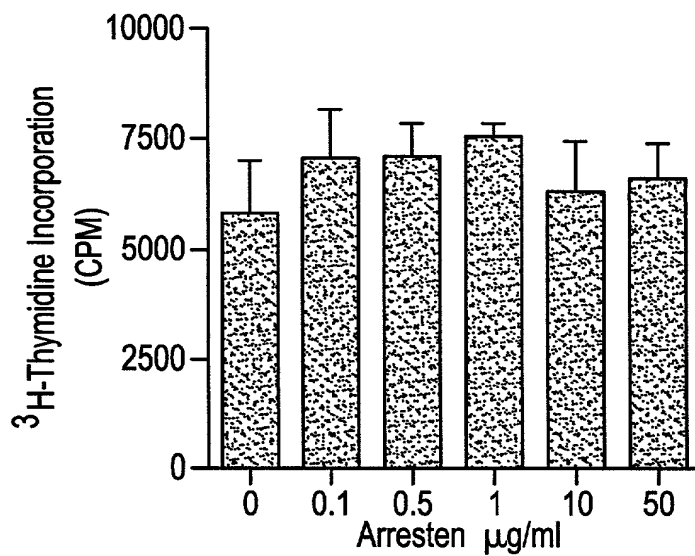


FIG. 4C

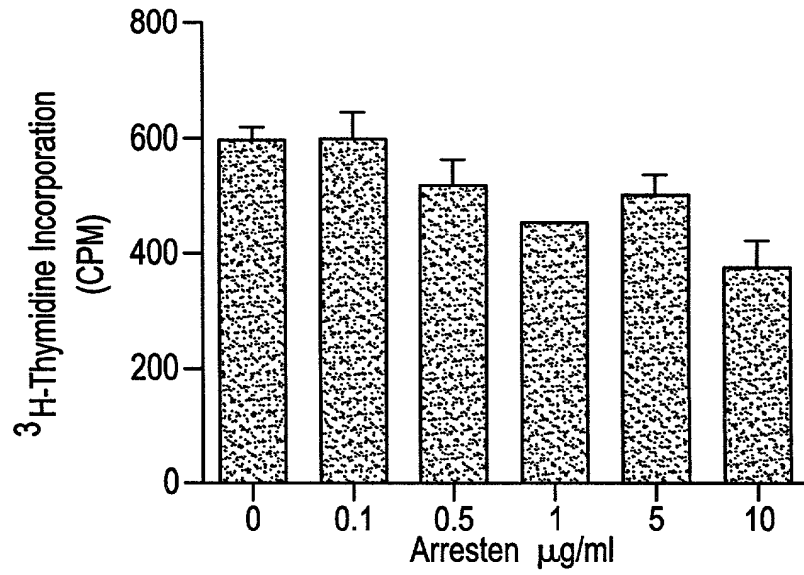


FIG. 4D

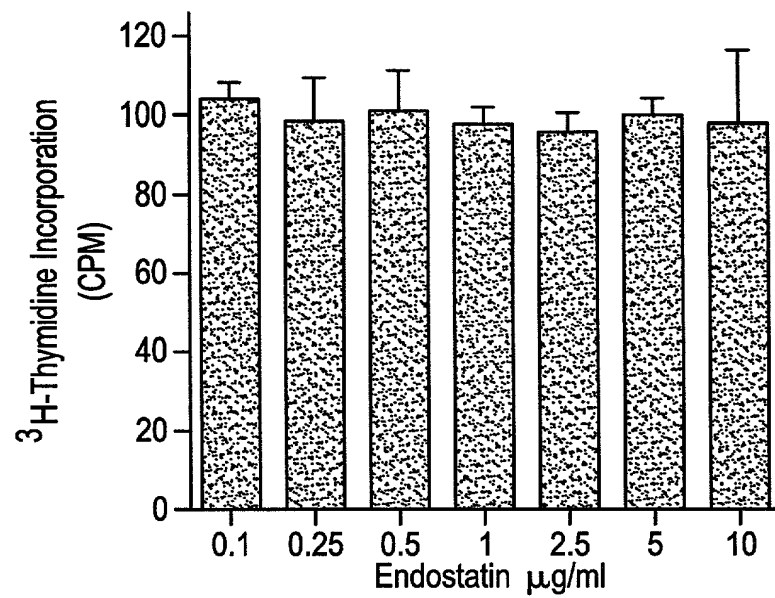


FIG. 5A

Control

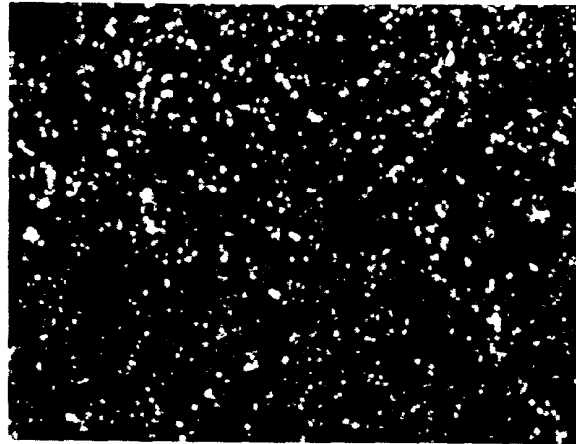


FIG. 5B

Arresten 2 μ g/ml

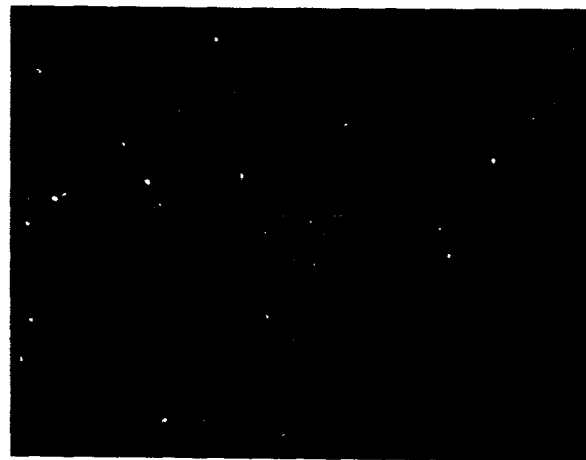


FIG. 5C

Endostatin 20 μ g/ml

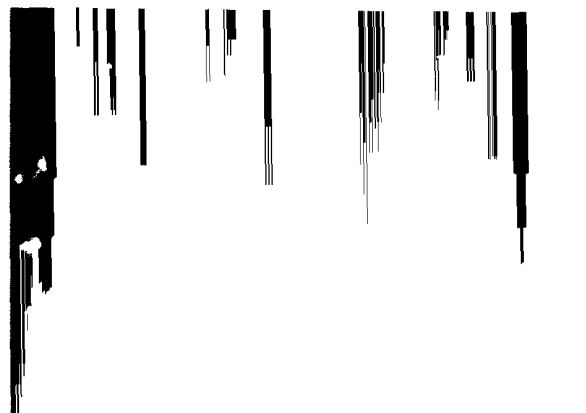


FIG. 6

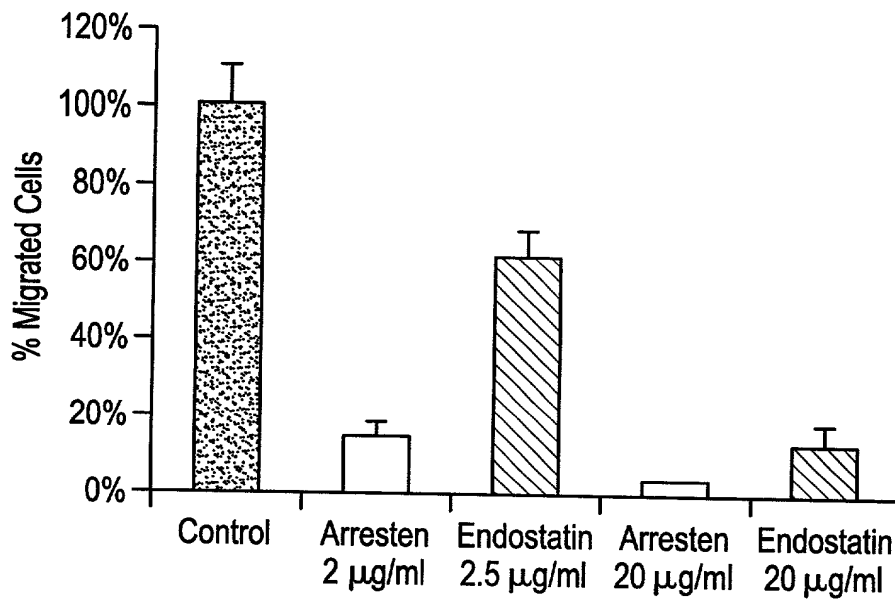


FIG. 7

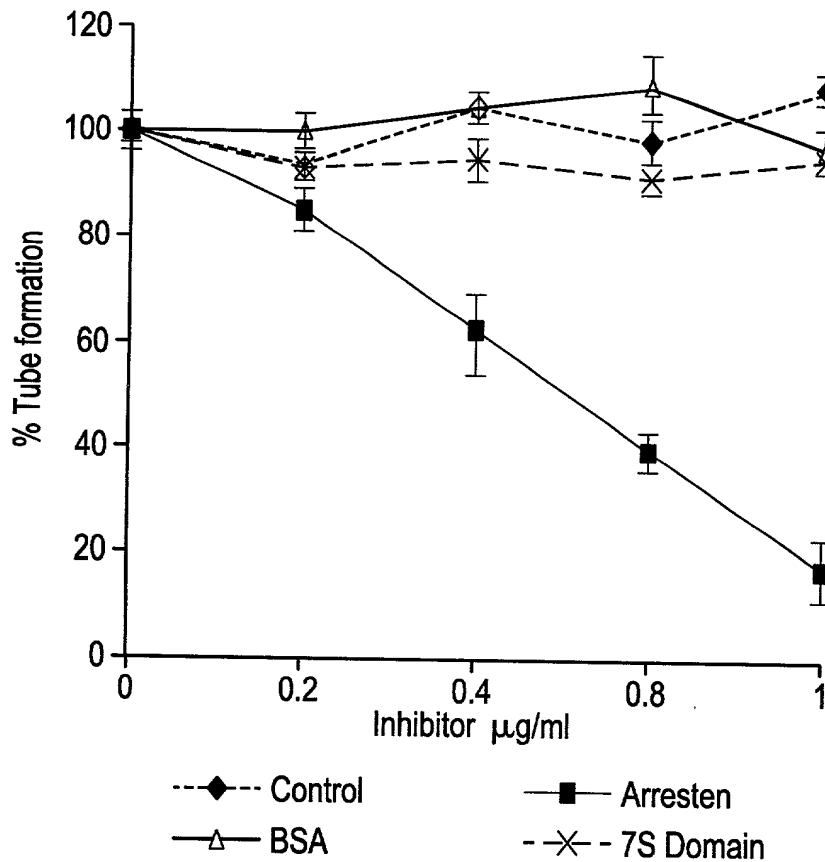


FIG. 8A

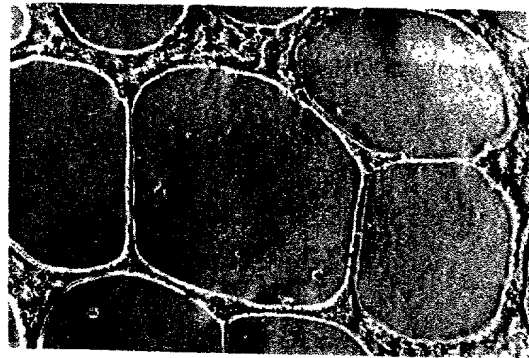


FIG. 8B



FIG. 9A

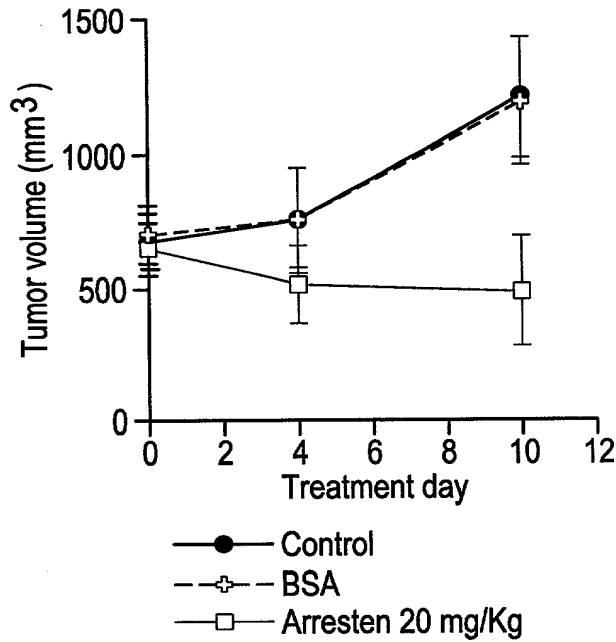


FIG. 9B

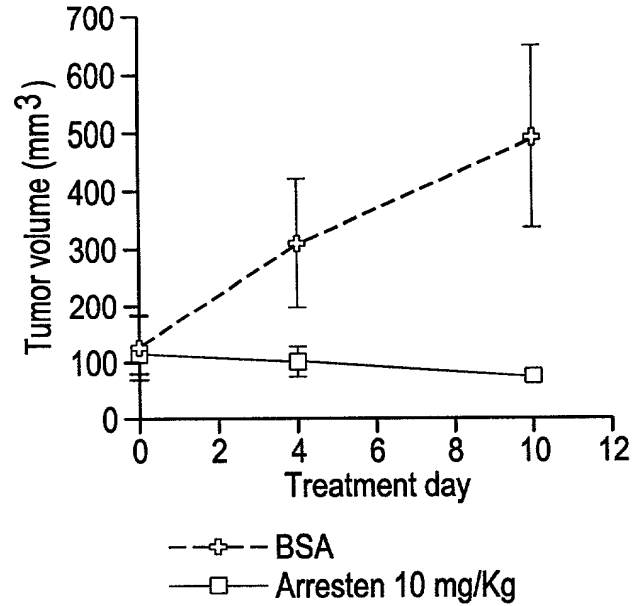


FIG. 9C

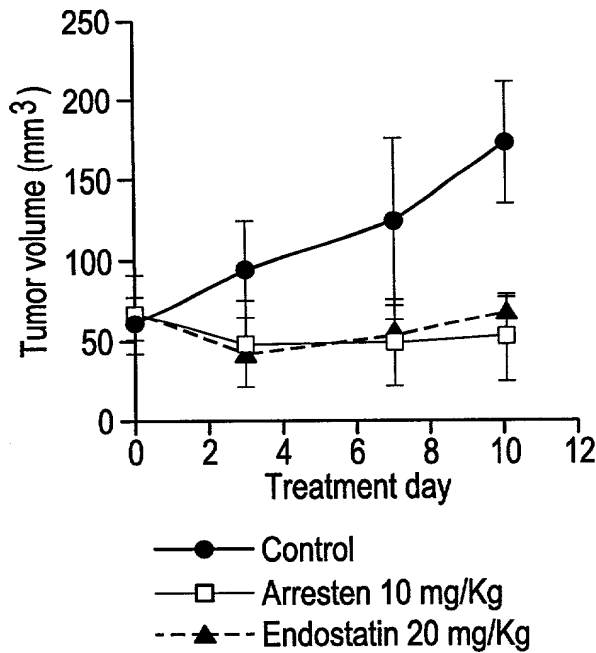
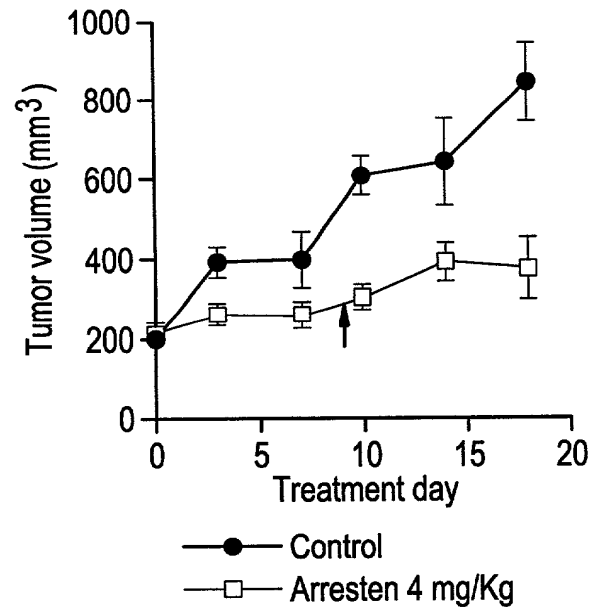


FIG. 9D



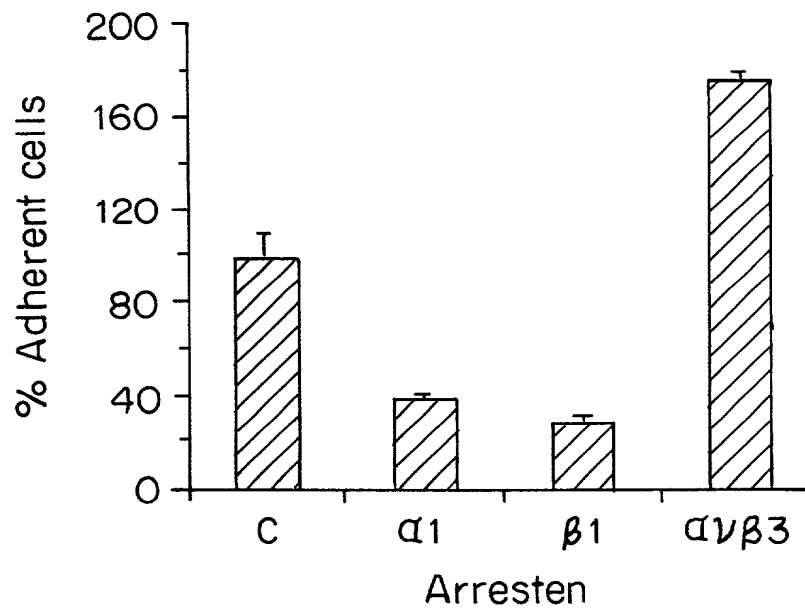


FIG. IOA

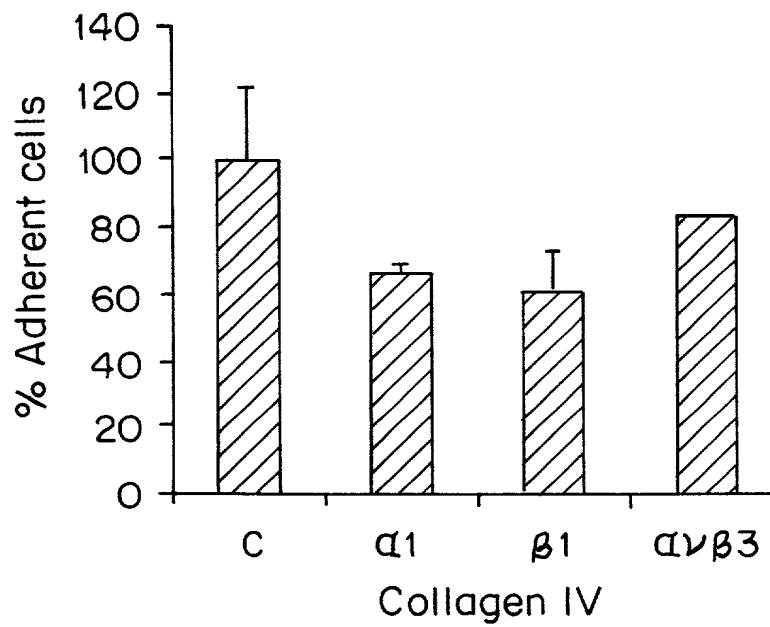
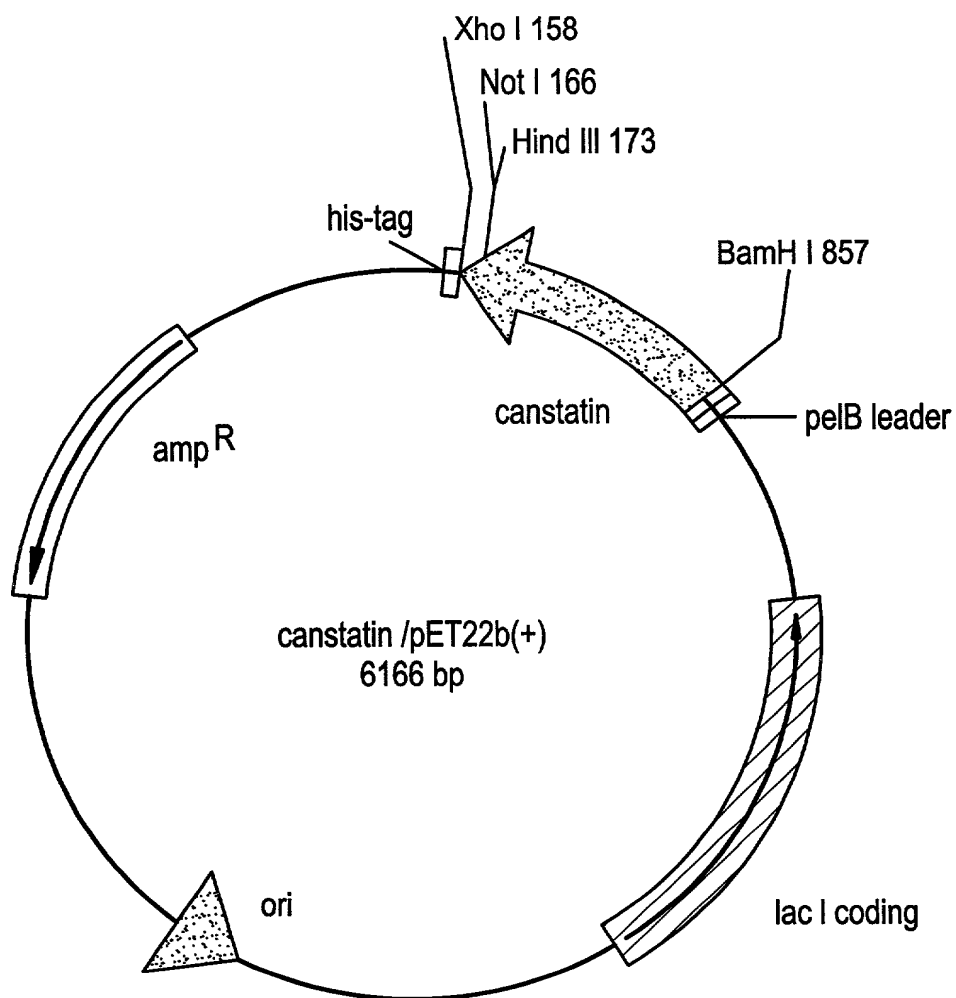


FIG. IOB

NL (SEQ ID NO:6)

FIG. 12



Forward primer: 5'-cgggatcctgtcagcatcggtacctc-3'

Reverse primer: 5'-cccaagcttcaggttctcatgcacac-3'

FIG. 13A

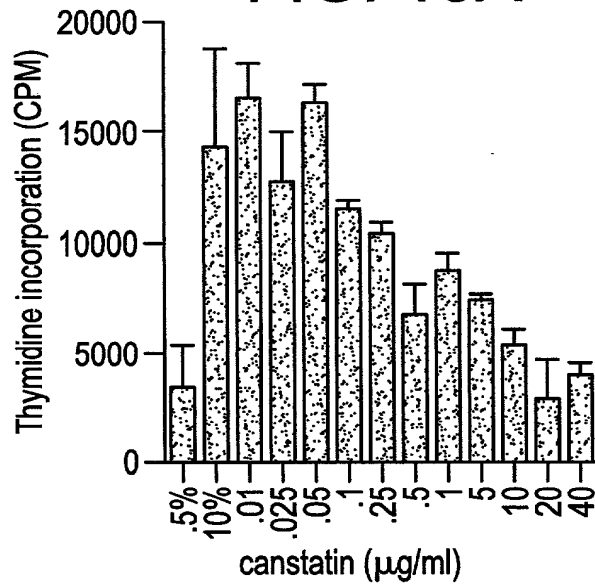
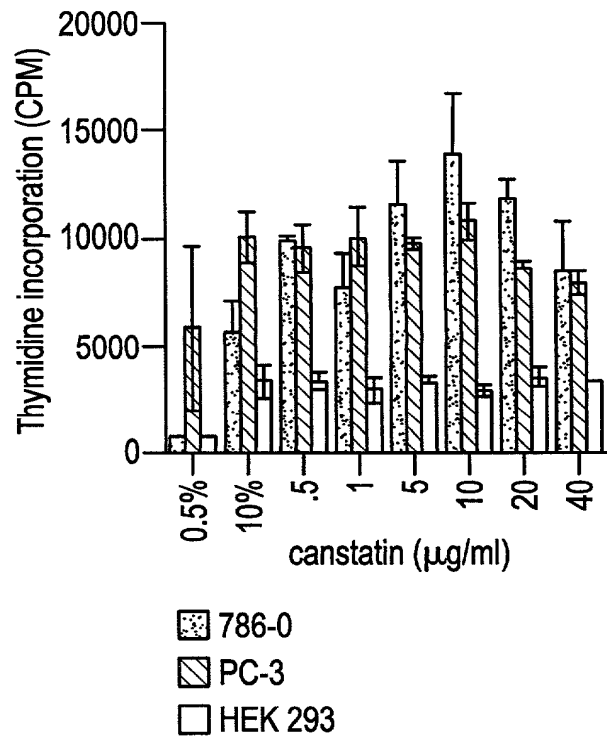


FIG. 13B



786-0
 PC-3
 HEK 293

FIG. 13C

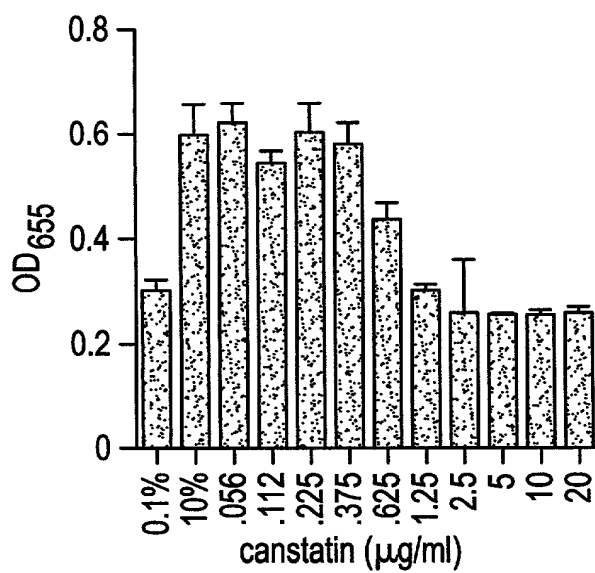
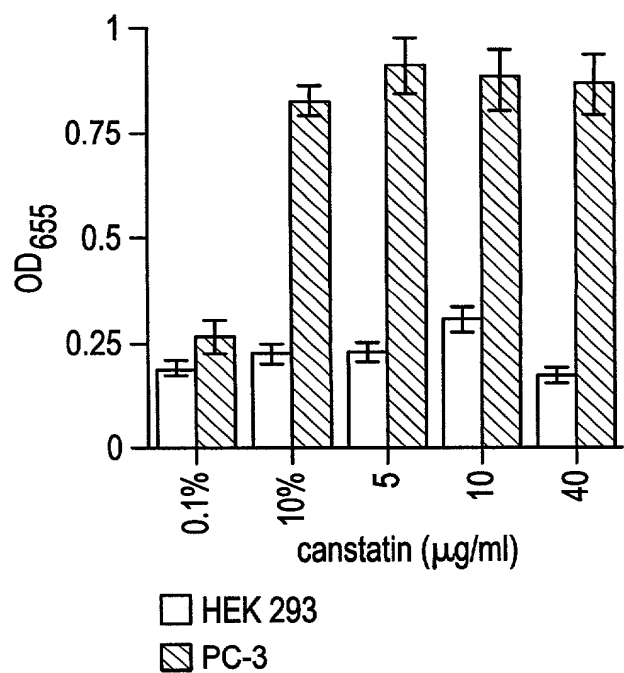


FIG. 13D



HEK 293
 PC-3

FIG. 14

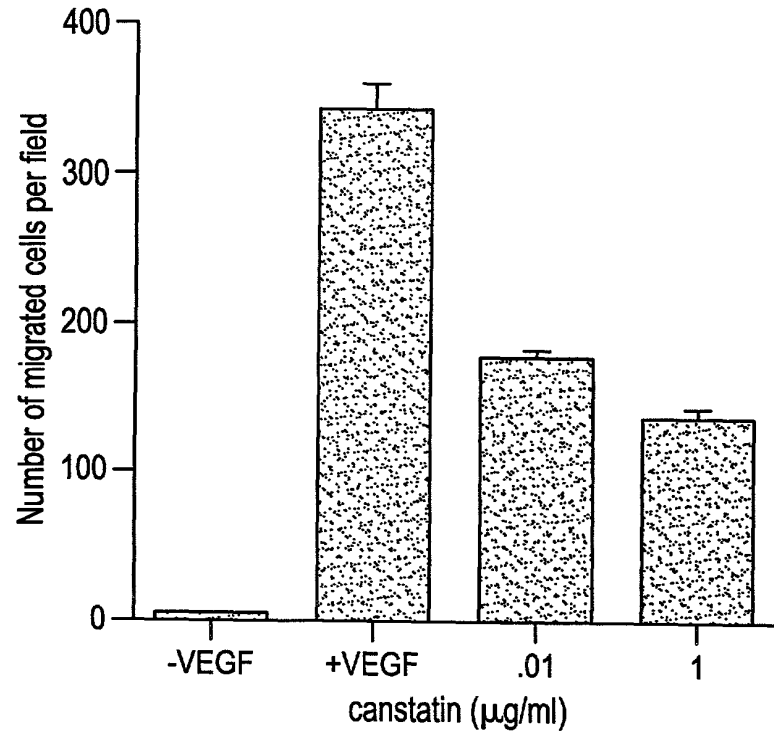
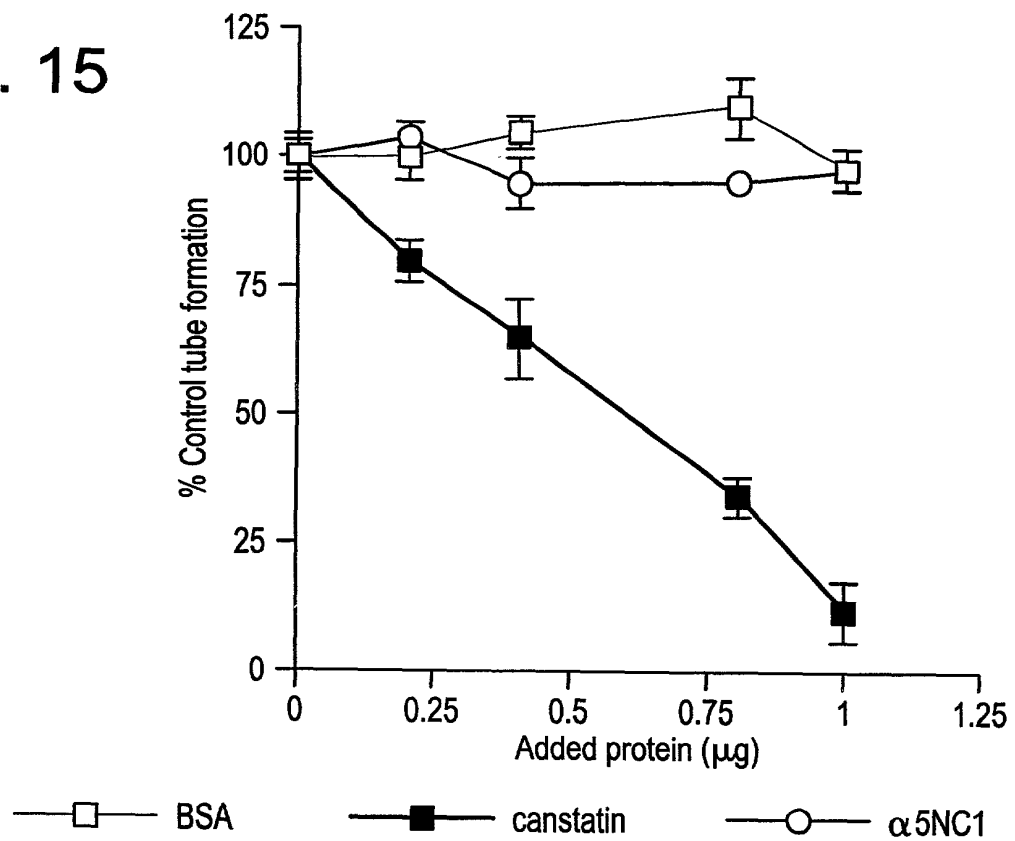


FIG. 15



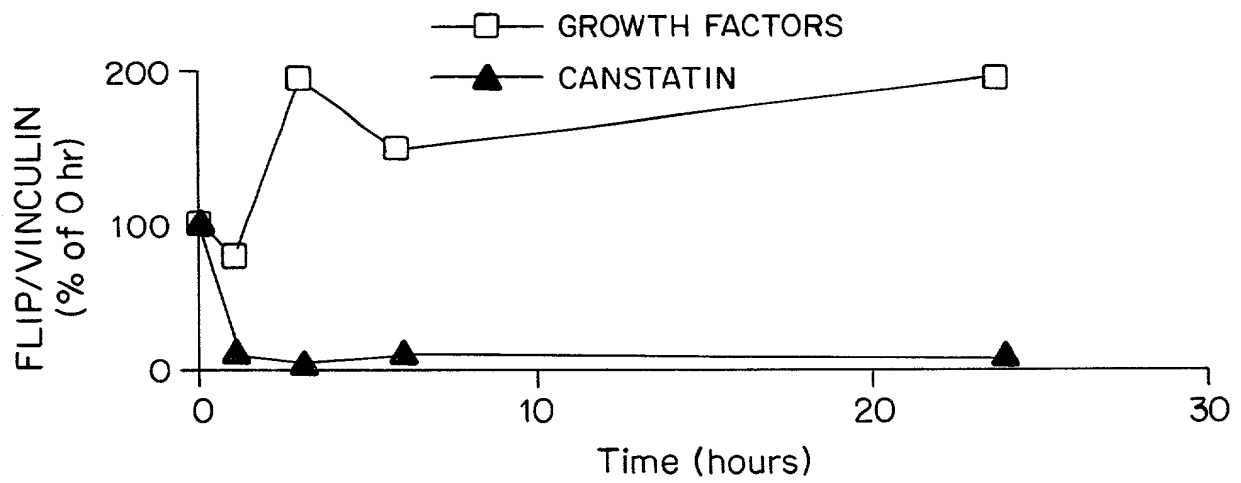


FIG. 16

FIG. 17A

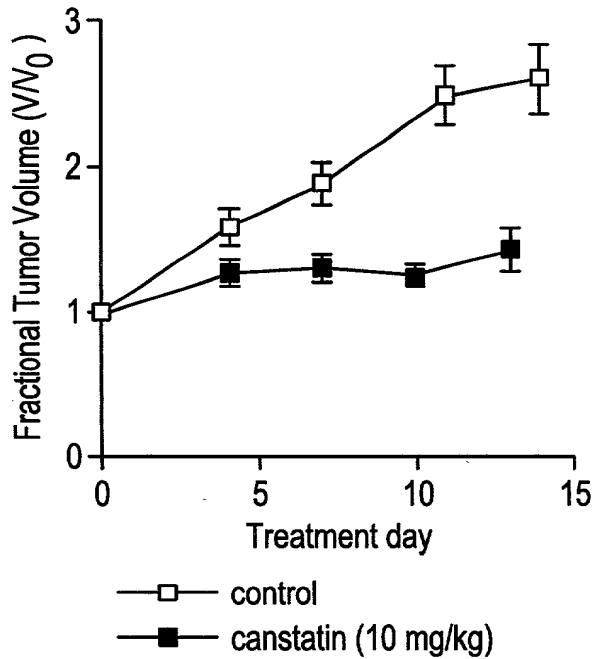


FIG. 17B

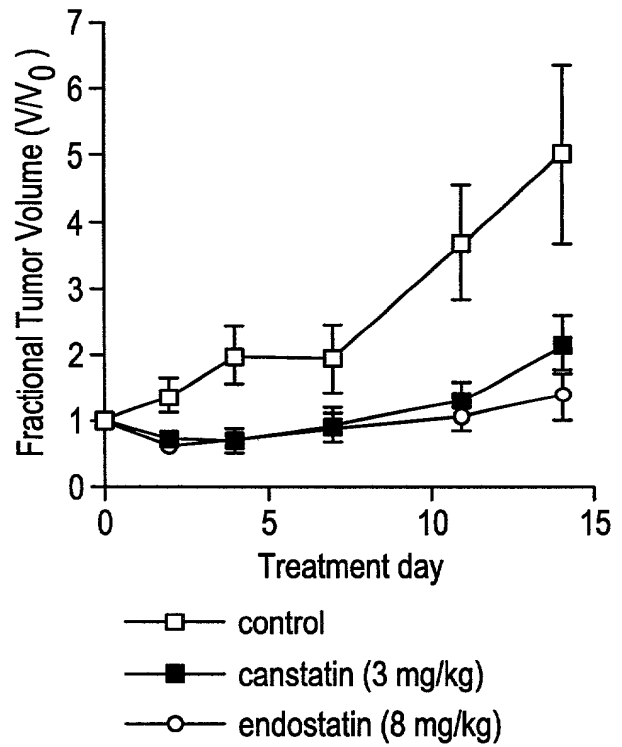


FIG. 17C

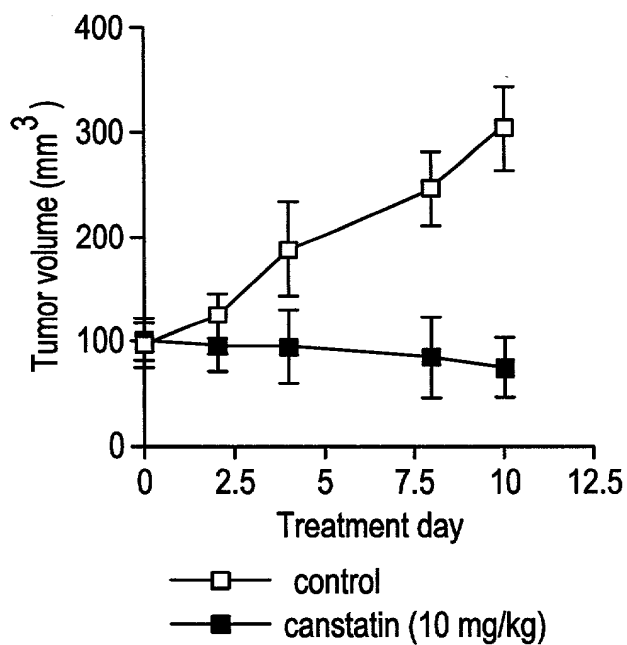


FIG. 17D

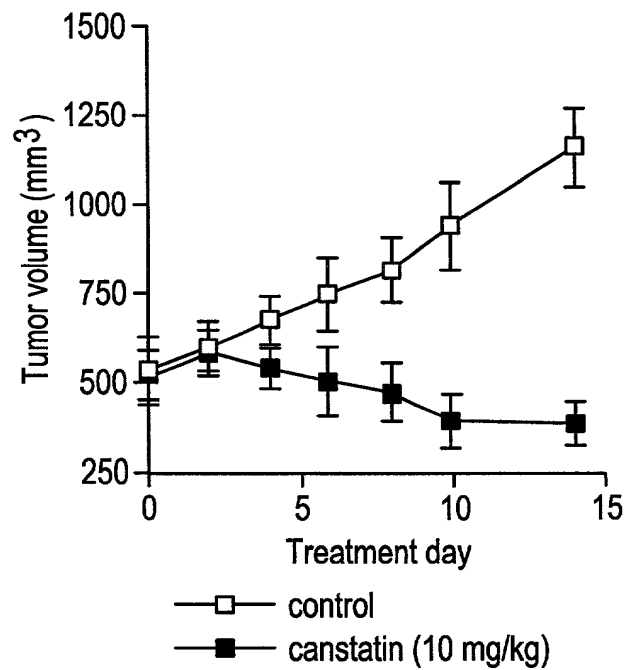


FIG. 18A

pET22b(+) forward primer:

5'-CGGGAT CCA GGT TTG AAA GGA AAA CGT-3' (SEQ ID NO:11)

pET22b(+) reverse primer:

5'-CCCAAGCTT TCA GTG TCT TTT CTT CAT-3' (SEQ ID NO:12)

| | | | | | | | | |
|------------------------------------|-------------|-------------|-------------|---------|---------|-----|-----|-----|
| 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 |
| <u>cca ggt ttg aaa gga aaa cgt</u> | gga gac agt | gga tca cct | gca acc | | | | | |
| 50 | 55 | 60 | 65 | 70 | 75 | 80 | 85 | 90 |
| tgg aca acg | aga ggc ttt | gtc ttc acc | cga cac agt | caa acc | aca | | | |
| 95 | 100 | 105 | 110 | 115 | 120 | 125 | 130 | 135 |
| gca att cct | tca tgt cca | gag ggg aca | gtg cca | ctc tac | agt ggg | | | |
| 140 | 145 | 150 | 155 | 160 | 165 | 170 | 175 | 180 |
| ttt tct ttt | ctt ttt gta | caa gga aat | caa cga | gcc cac | gga caa | | | |
| 185 | 190 | 195 | 200 | 205 | 210 | 215 | 220 | 225 |
| gac ctt gga | act ctt ggc | agc tgc ctg | cag cga | ttt acc | aca atg | | | |
| 230 | 235 | 240 | 245 | 250 | 255 | 260 | 265 | 270 |
| cca ttc tta | ttc tgc aat | gtc aat gat | gta tgt | aat ttt | gca tct | | | |
| 275 | 280 | 285 | 290 | 295 | 300 | 305 | 310 | 315 |
| cga aat gat | tat tca tac | tgg ctg tca | aca cca | gct ctg | atg cca | | | |
| 320 | 325 | 330 | 335 | 340 | 345 | 350 | 355 | 360 |
| atg aac atg | gct ccc att | act ggc | aga gcc | ctt gag | cct tat | ata | | |
| 365 | 370 | 375 | 380 | 385 | 390 | 395 | 400 | 405 |
| agc aga tgc | act gtt tgt | gaa ggt cct | gcg atc | gcc ata | gcc gtt | | | |
| 410 | 415 | 420 | 425 | 430 | 435 | 440 | 445 | 450 |
| cac agc caa | acc act gac | att cct cca | tgt cct | cac ggc | tgg att | | | |
| 455 | 460 | 465 | 470 | 475 | 480 | 485 | 490 | 495 |
| tct ctc tgg | aaa gga ttt | tca ttc | atc atg | ttc aca | agt gca | ggt | | |
| 500 | 505 | 510 | 515 | 520 | 525 | 530 | 535 | 540 |
| tct gag ggc | acc ggg caa | gca ctg | gcc tcc | cct ggc | tcc tgc | ctg | | |
| 545 | 550 | 555 | 560 | 565 | 570 | 575 | 580 | 585 |
| gaa gaa ttc | cga gcc agc | cca ttt | cta gaa | tgt cat | gga aga | gga | | |
| 590 | 595 | 600 | 605 | 610 | 615 | 620 | 625 | 630 |
| acg tgc aac | tac tat tca | aat tcc | tac agt | ttc tgg | ctg gct | tca | | |
| 635 | 640 | 645 | 650 | 655 | 660 | 665 | 670 | 675 |
| tta aac cca | gaa aga atg | ttc aga | aag cct | att cca | tca act | gtg | | |
| 680 | 685 | 690 | 695 | 700 | 705 | 710 | 715 | 720 |
| aaa gct ggg | gaa tta gaa | aaa ata | ata agt | cgc tgt | cag gtg | tgc | | |
| 725 | 730 | 735 | | | | | | |
| <u>atg aag aaa aga cac tga</u> | | | | | | | | |

(SEQ ID NO:9)

pET22b- α 3(IV) NC1 = nucleotides 4 through 735

Tumstatin 333 = nucleotides 4 through 375

Tumstatin 334 - nucleotide 376 through 735

FIG. 18B

*
5 10 15 20 25 30 35 40 45
PGL KGK RGD SGS PAT WTT RGF VFT RHS QTT AIP SCP EGT VPL YSG
50 55 60 65 70 75 80 85 90
FSF LFV QGN QRA HGQ DLG TLG SCL QRF TTM PFL FCN VND VCN FAS
*+
95 100 105 110 115 120 125 130 135
RND YSY WLS TPA LMP MNM API TGR ALE PYI SRC TVC EGP AIA IAV
140 145 150 155 160 165 170 175 180
HSQ TTD IPP CPH GWI SLW KGF SFI MFT SAG SEG TGQ ALA SPG SCL
185 190 195 200 205 210 215 220 225
EEF RAS PFL ECH GRG TCN YYS NSY SFW LAS LNP ERM FRK PIP STV
+
230 235 240 245
KAG ELE KII SRC QVC MKK RH (SEQ ID NO:10)

pET22b α 3(IV) NC1 = residues 2 through 245

Tumstatin 333 = residues 2 through 125

Tumstatin 334 = residues 126 through 245

FIG. 19

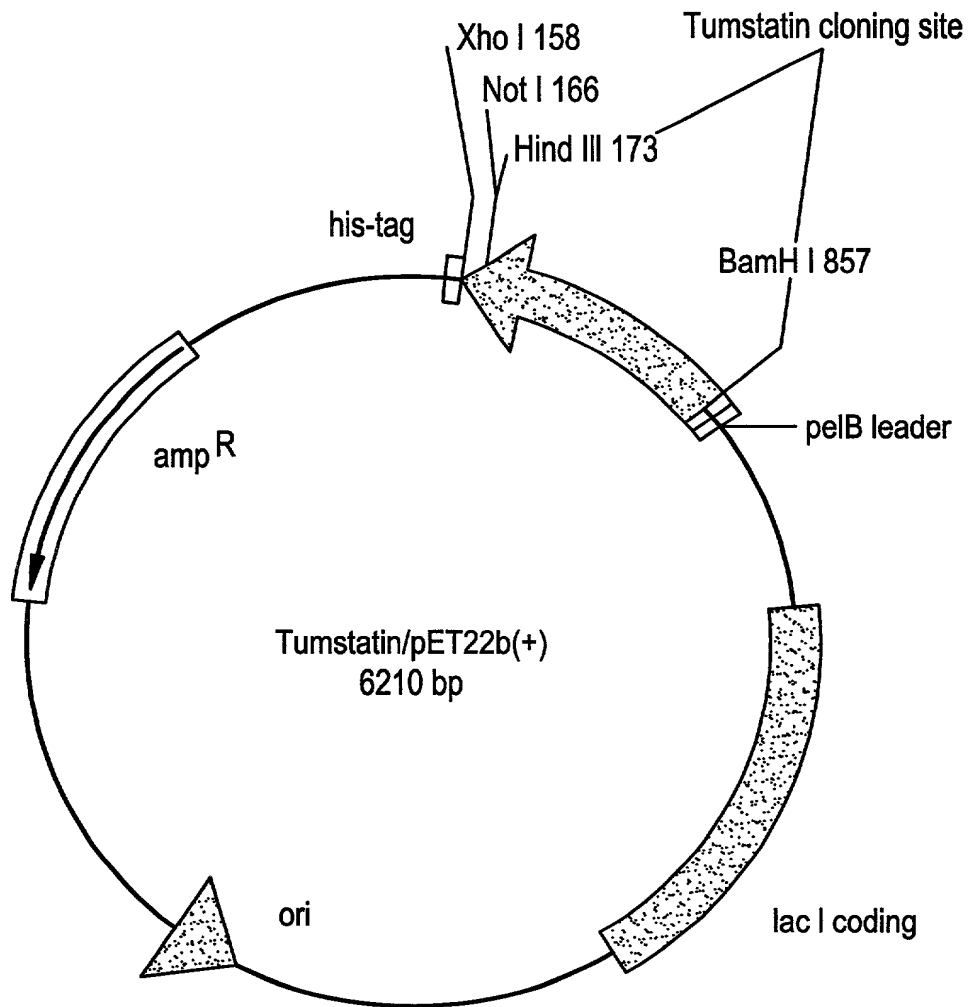
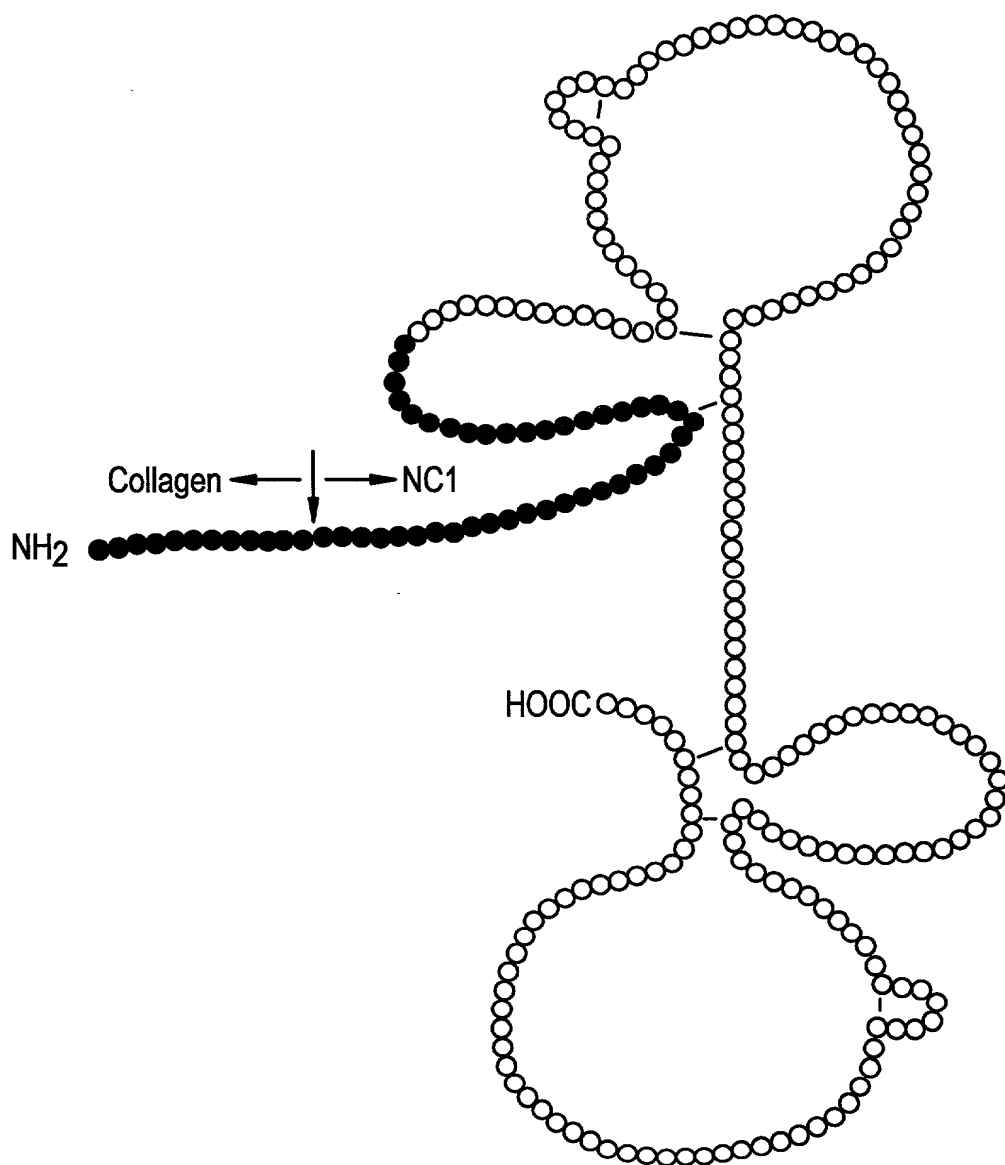
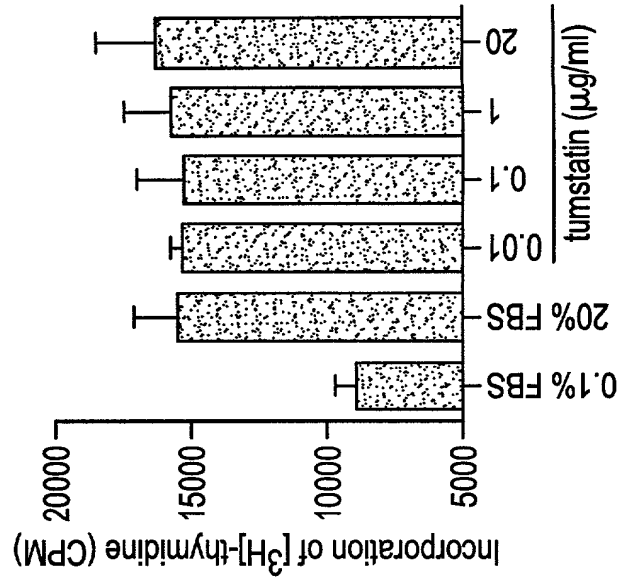
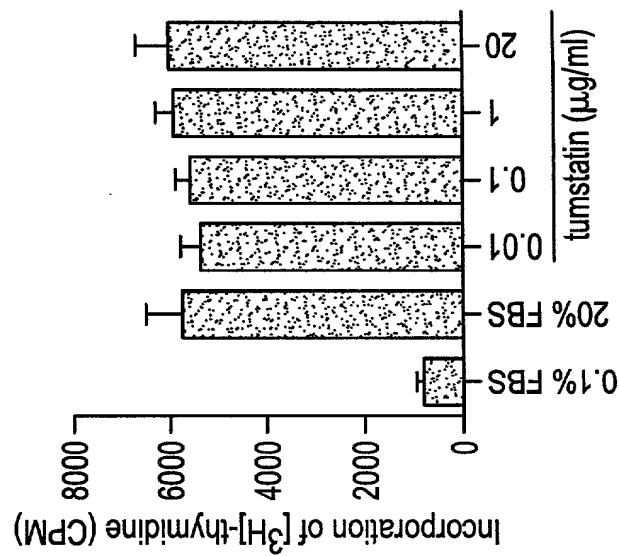
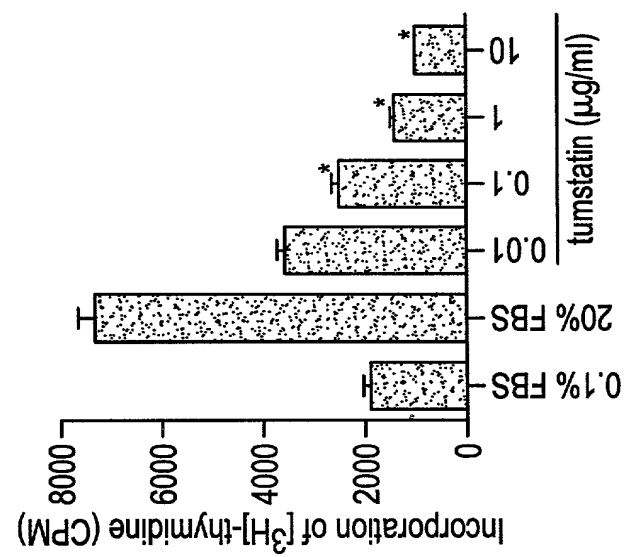


FIG. 20





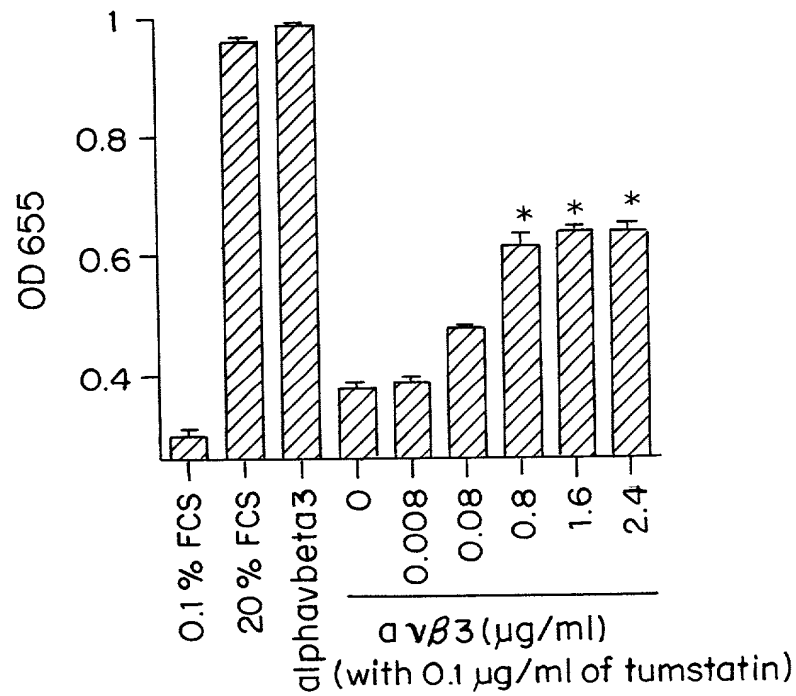


FIG. 22

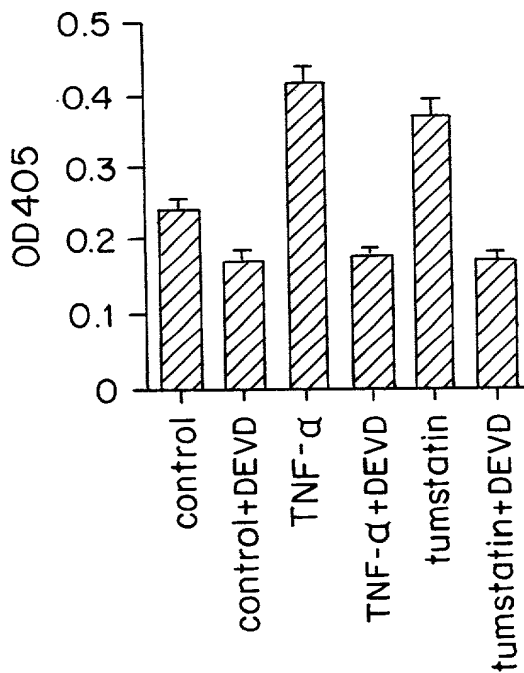


FIG. 23A

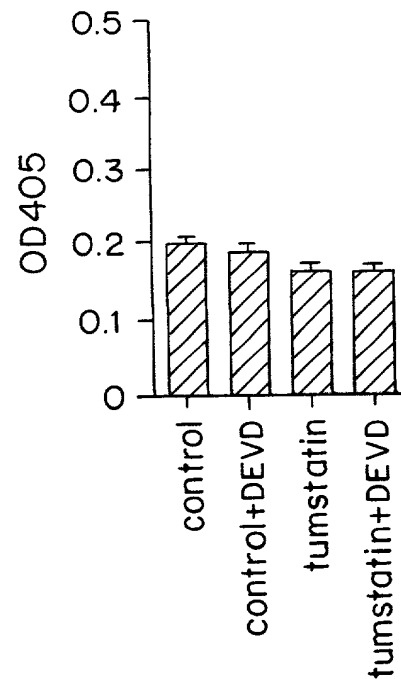


FIG. 23B

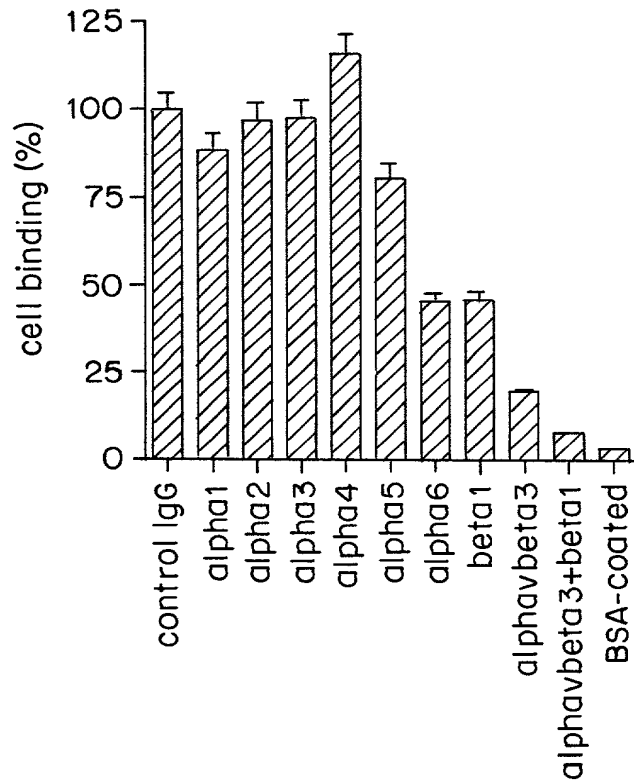


FIG. 24A

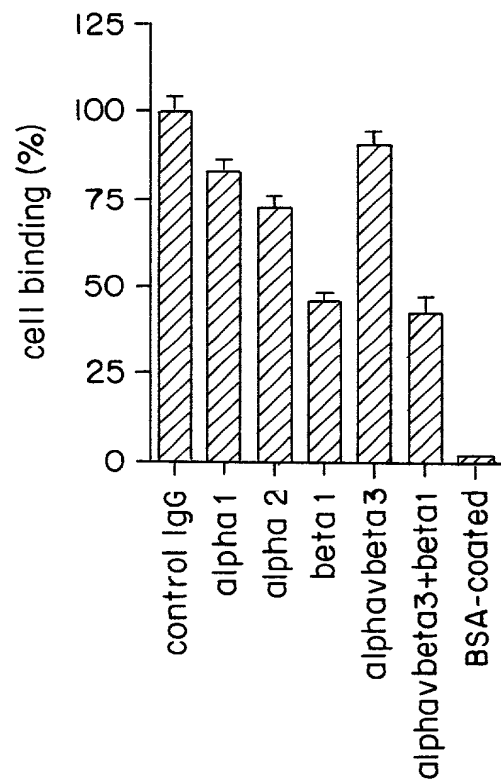


FIG. 24B

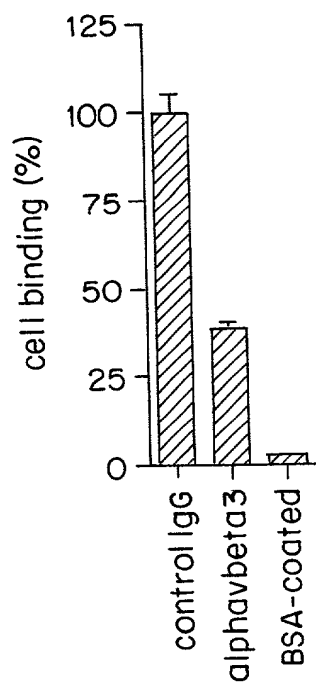


FIG. 24C

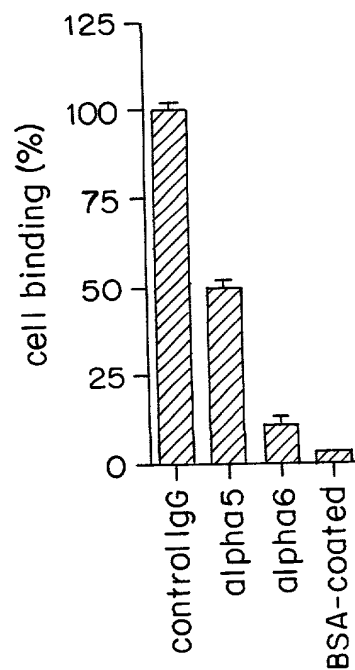


FIG. 24D

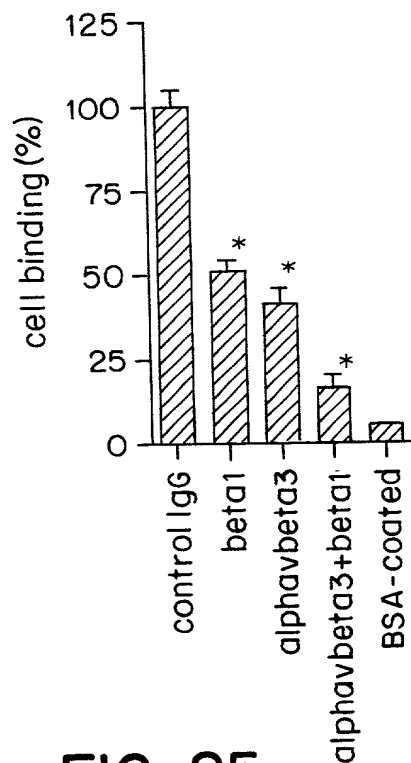


FIG. 25

FIG. 26

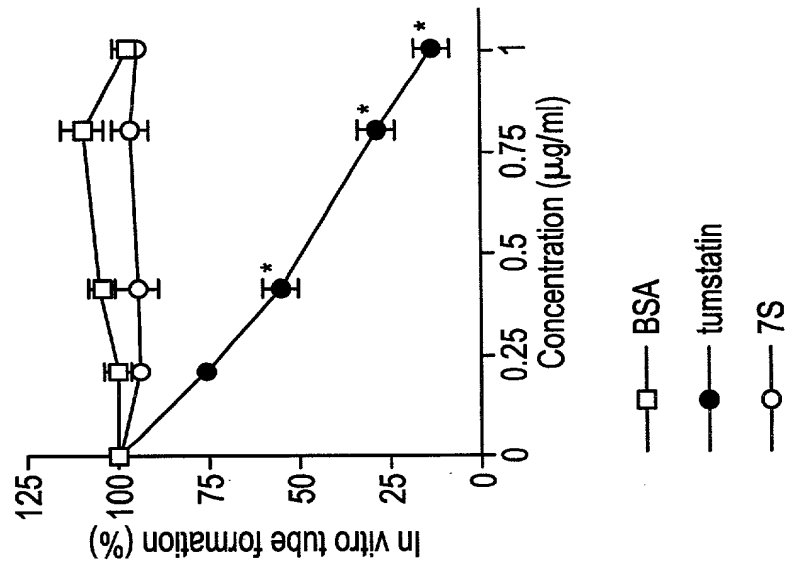


FIG. 27A

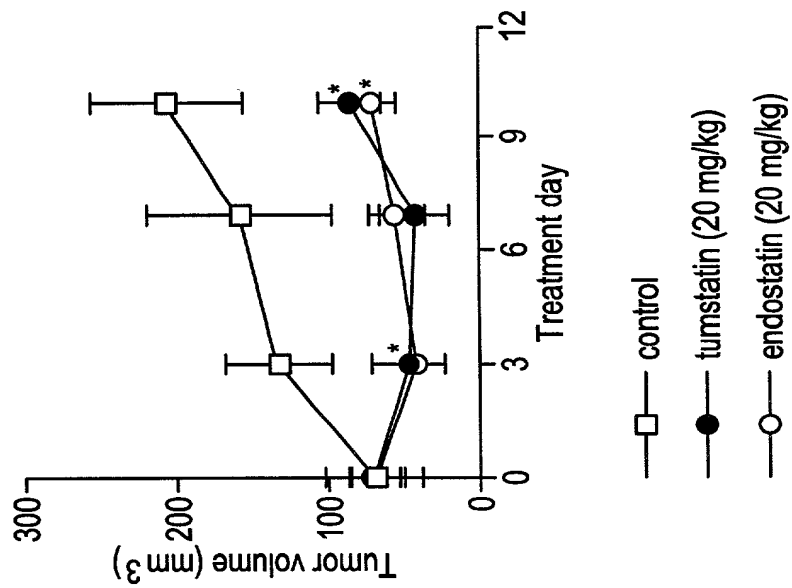


FIG. 27B

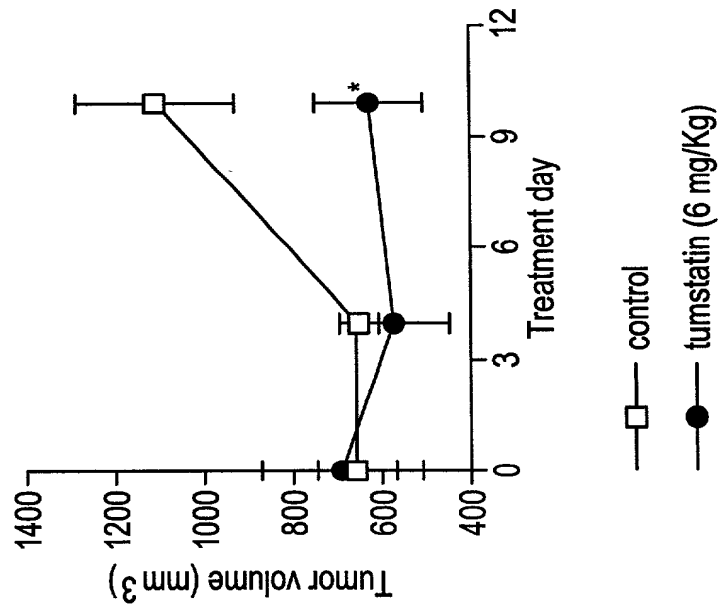
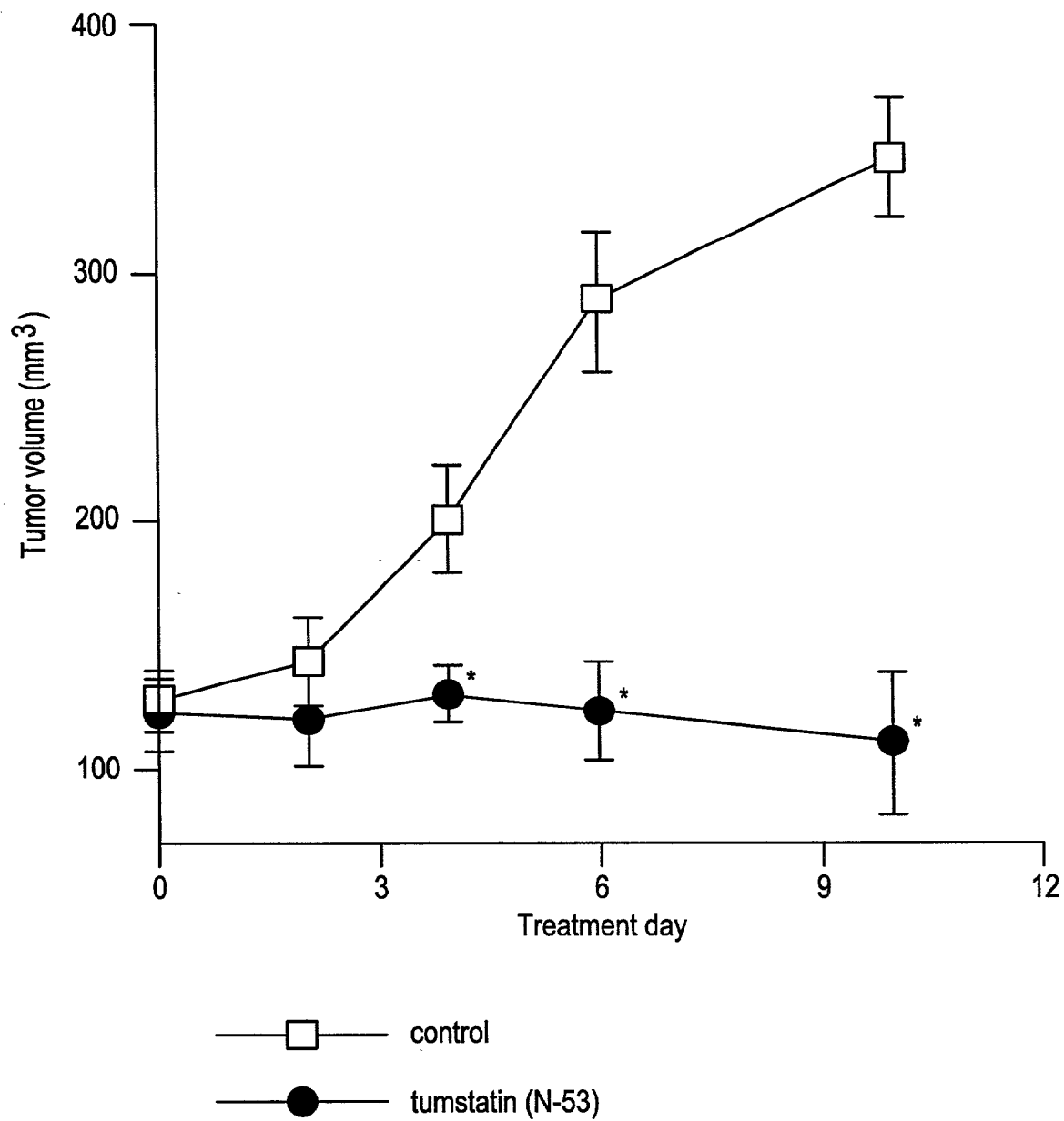


FIG. 28



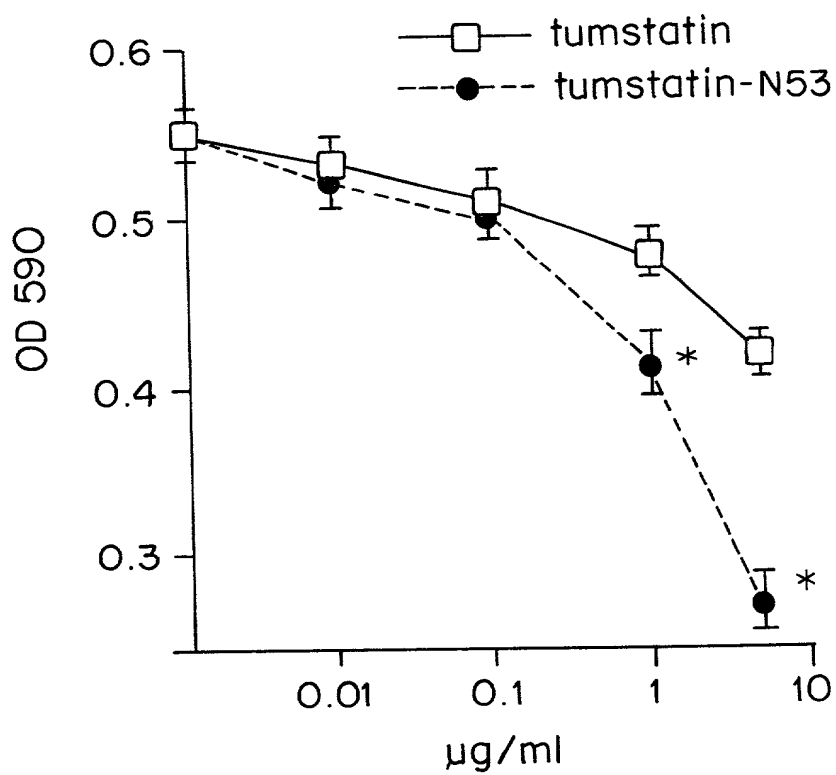
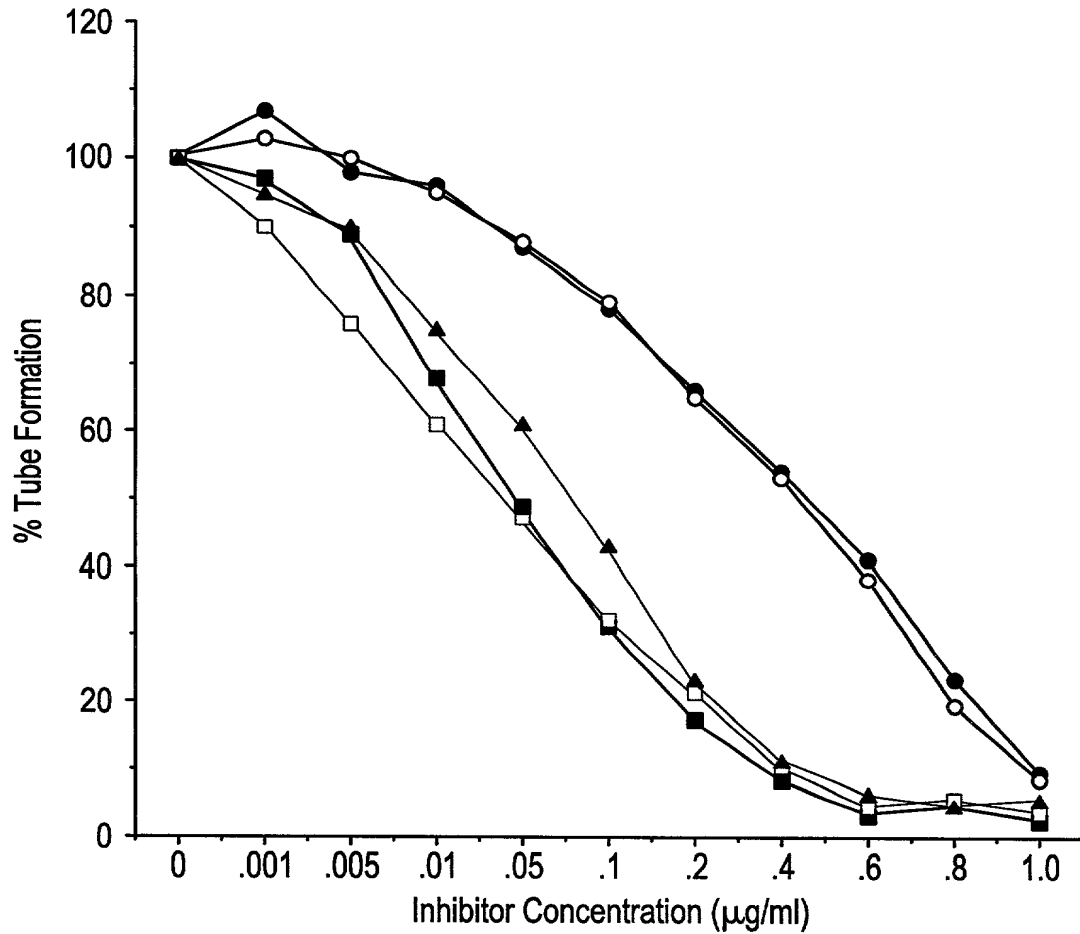


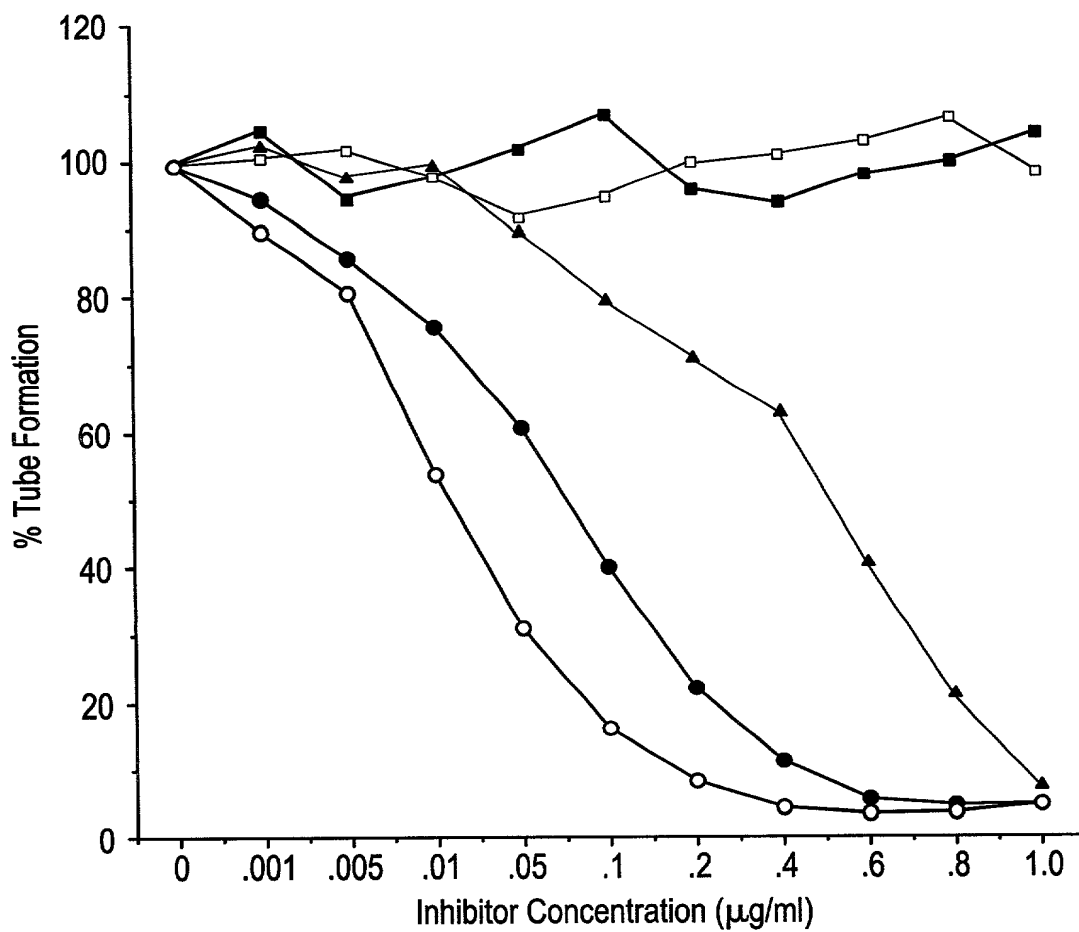
FIG. 29

FIG. 30



- Arresten
- Canstatin
- 12 kDa fragment of Arresten
- 8 kDa fragment of Arresten
- ▲— 10 kDa fragment of Canstatin

FIG. 31



- Tumstatin Fragment 333
- Tumstatin Fragment 334
- BSA
- α6
- ▲— Tumstatin

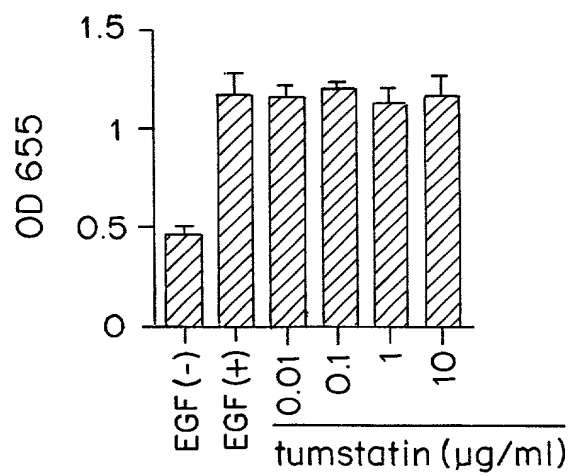


FIG. 32A

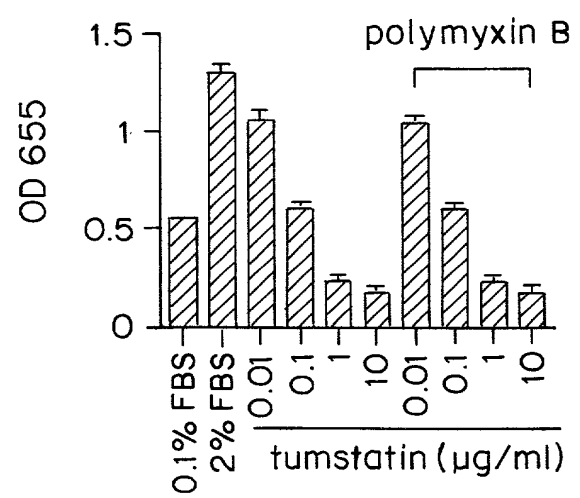


FIG. 32B

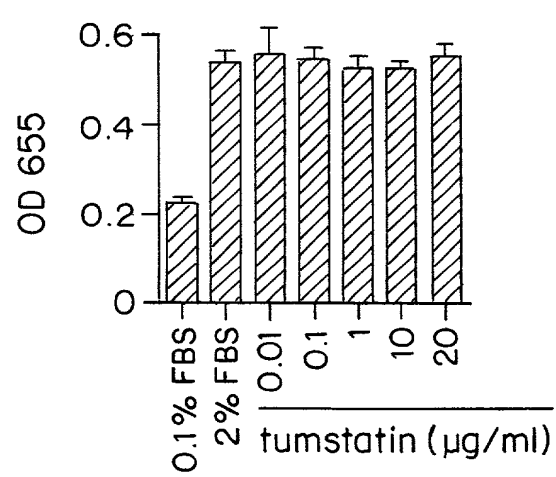


FIG. 32C

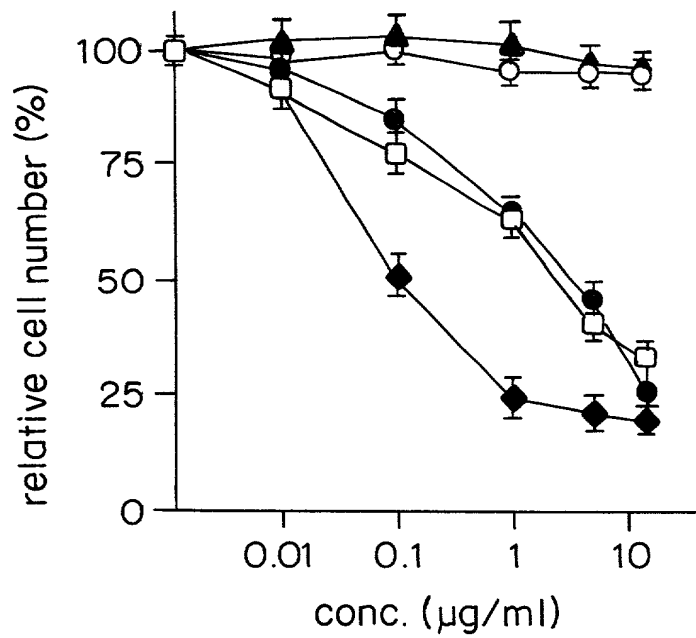


FIG. 33A

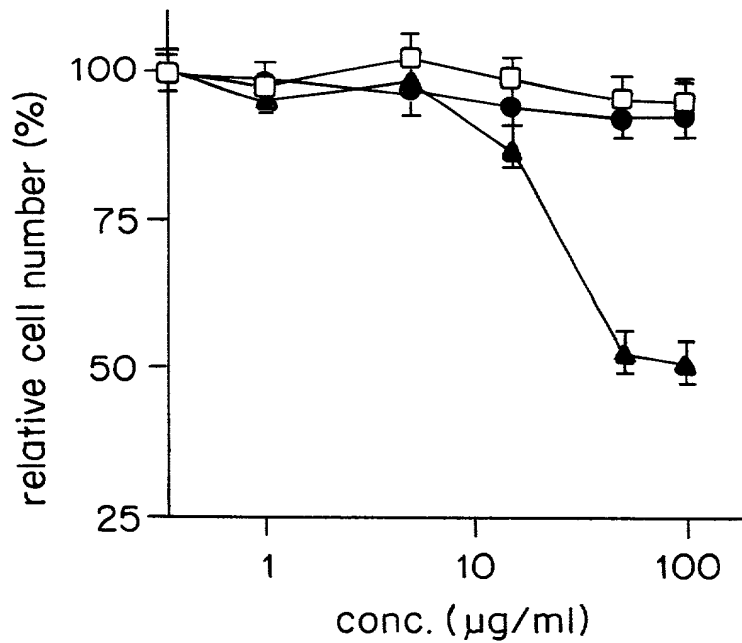


FIG. 33B

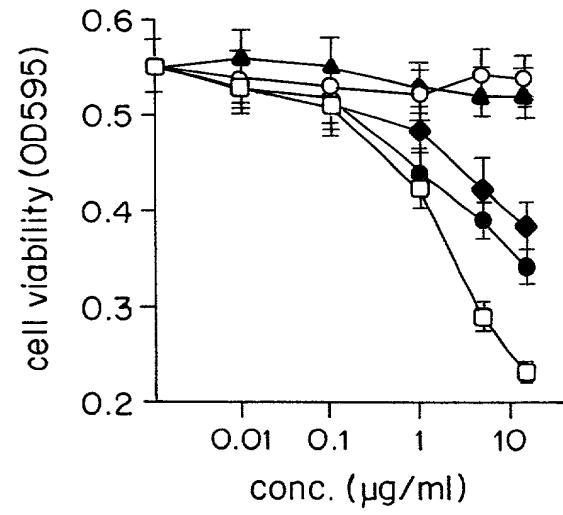


FIG. 34A

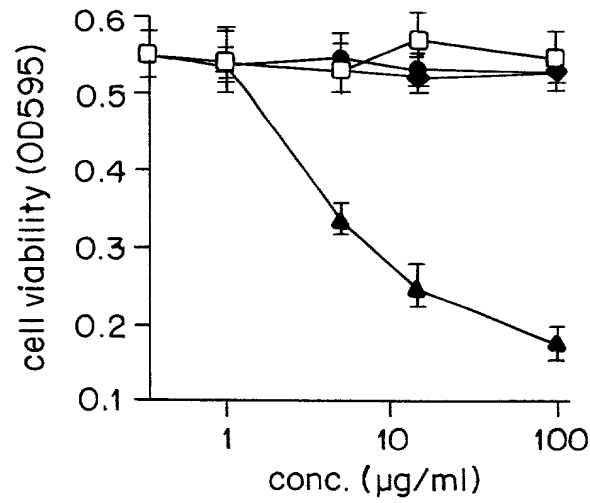


FIG. 34B

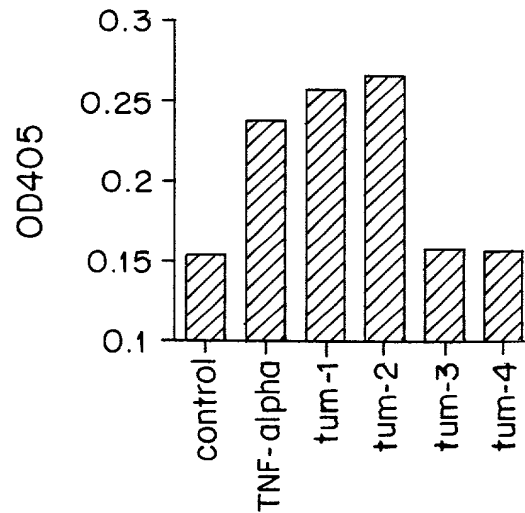


FIG. 35

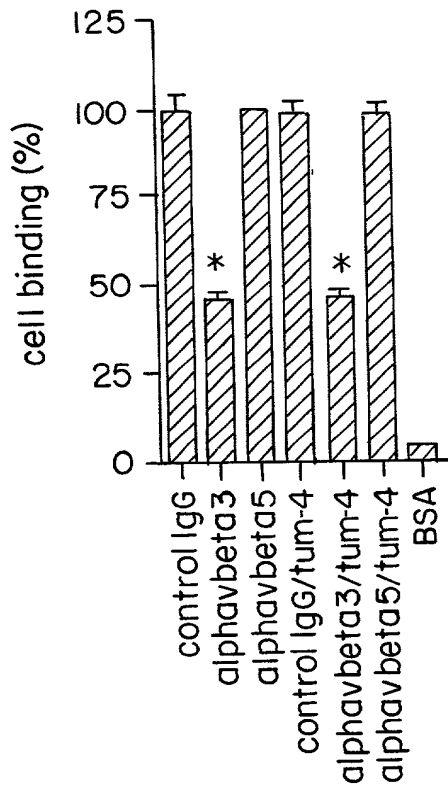


FIG. 36A

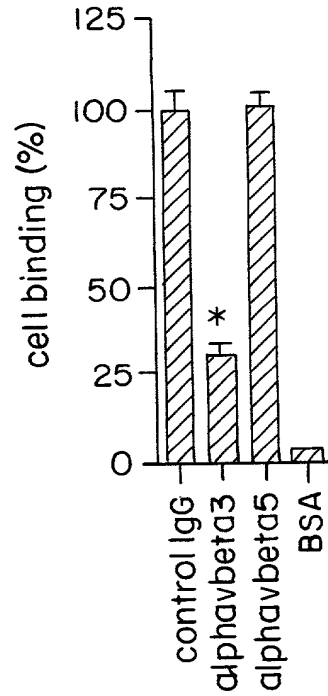


FIG. 36B

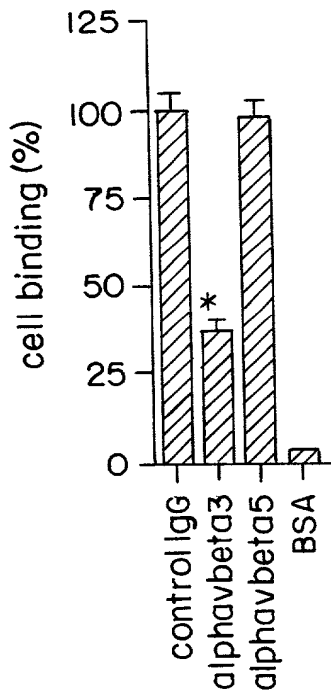


FIG. 36C

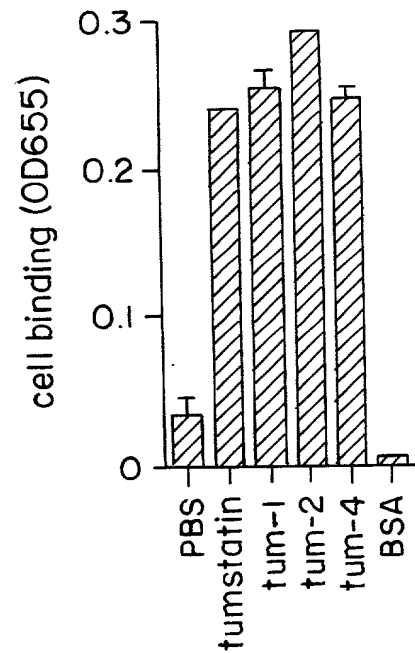


FIG. 37

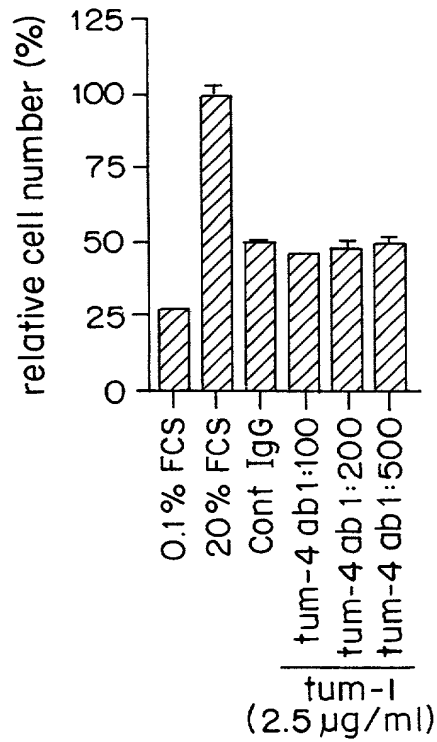


FIG. 38A

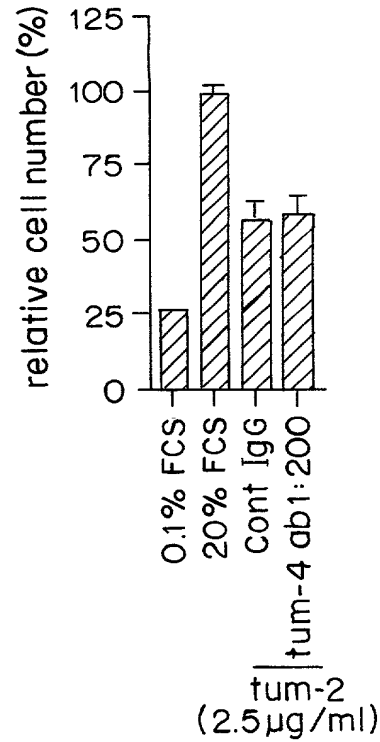


FIG. 38B

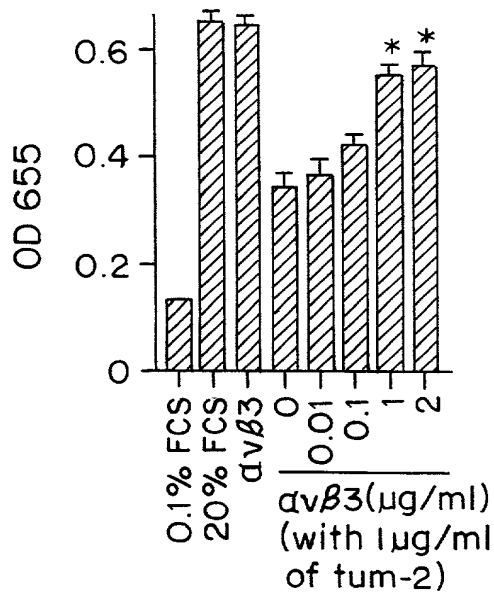


FIG. 38C

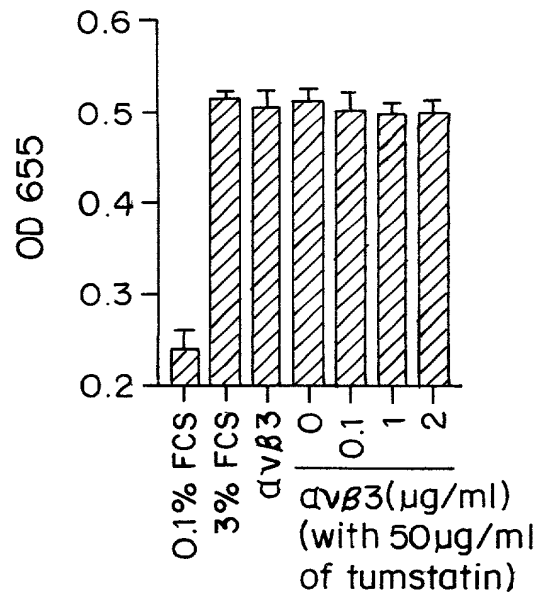


FIG. 38D

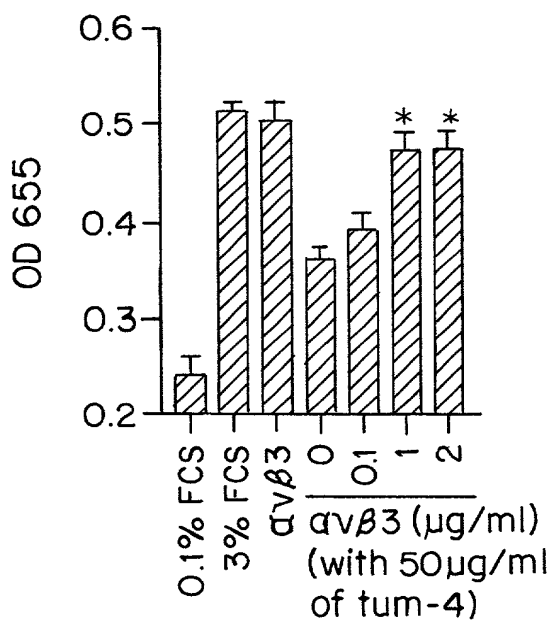


FIG. 38E

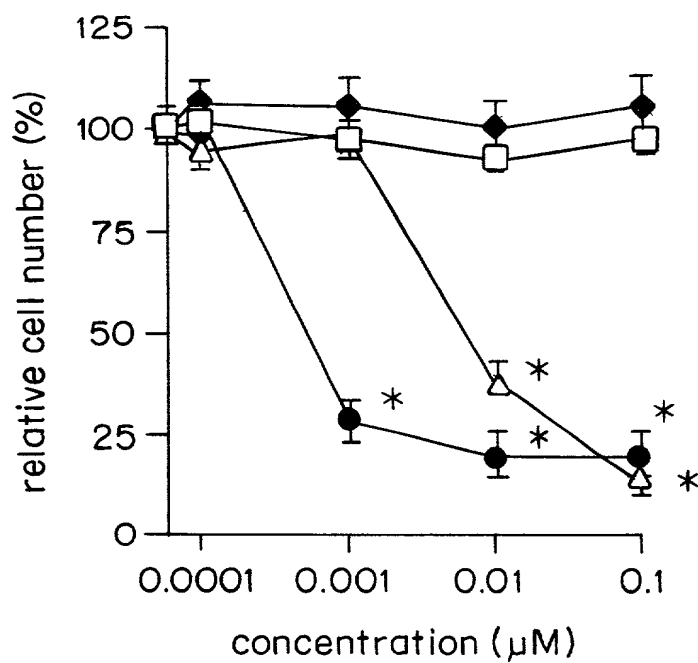


FIG. 39

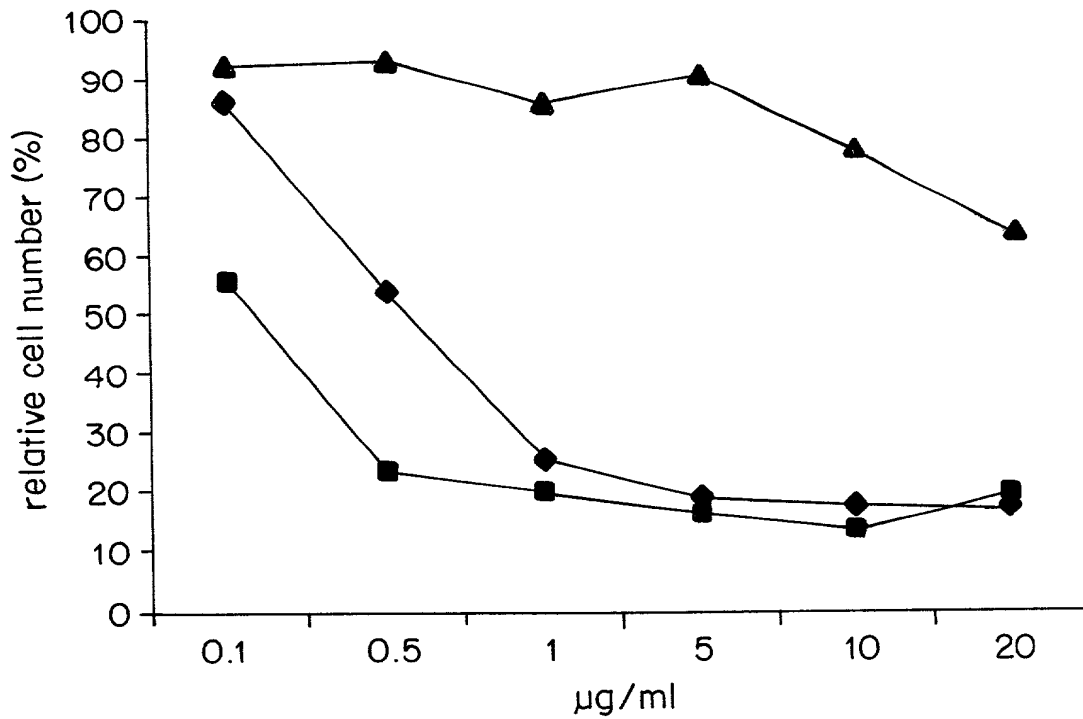


FIG. 40

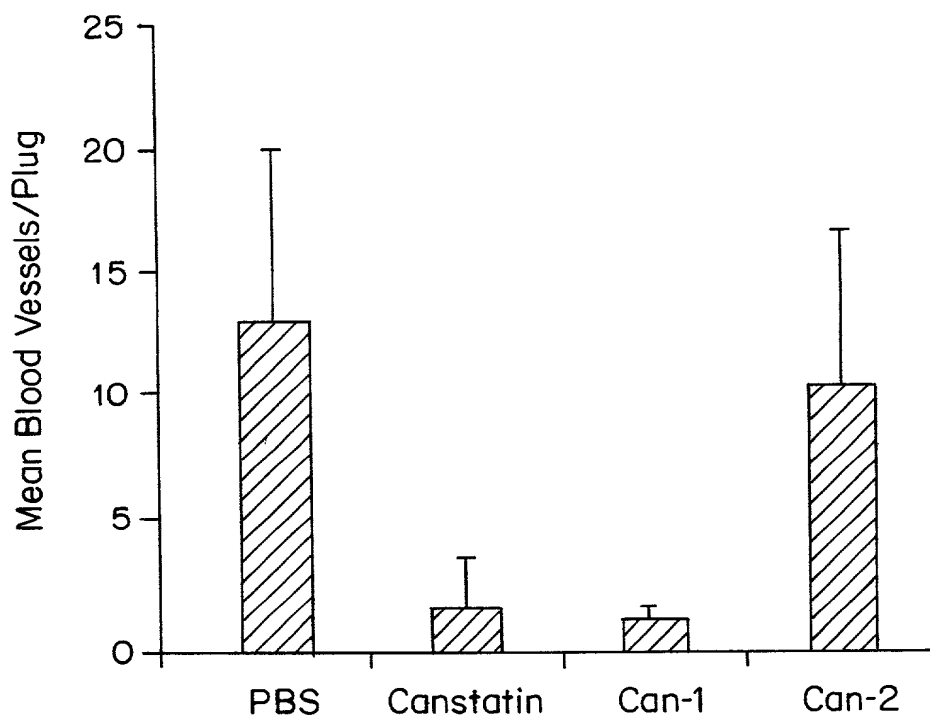


FIG. 41

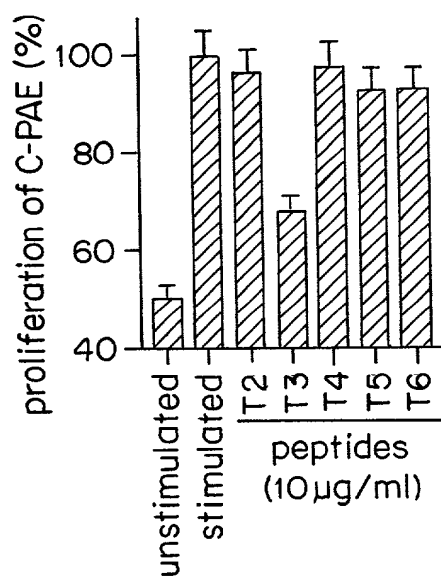


FIG. 43A

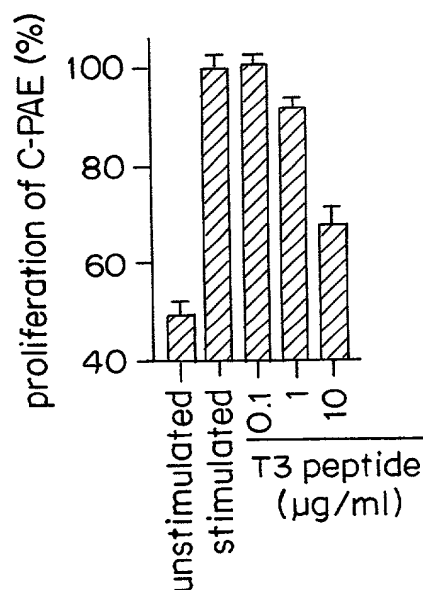


FIG. 43B

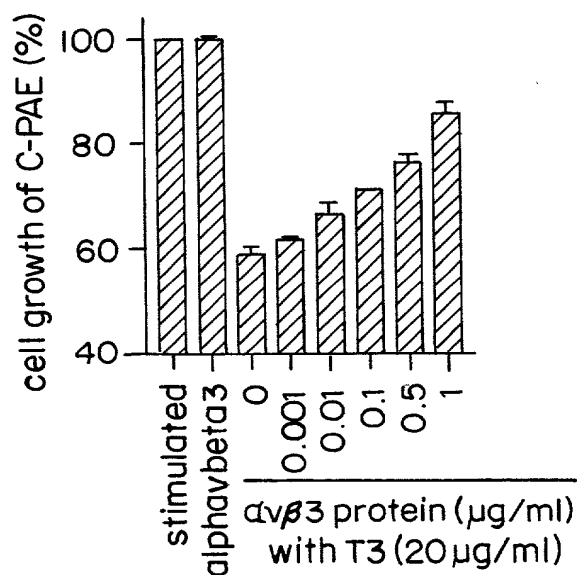


FIG. 43C

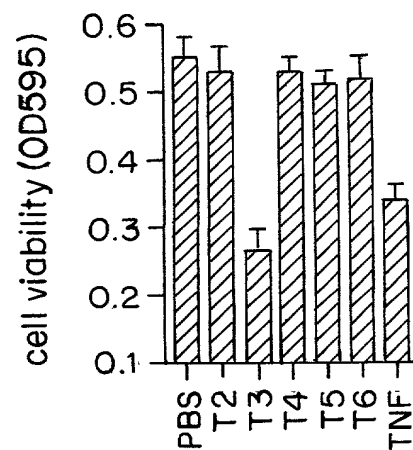


FIG. 43D

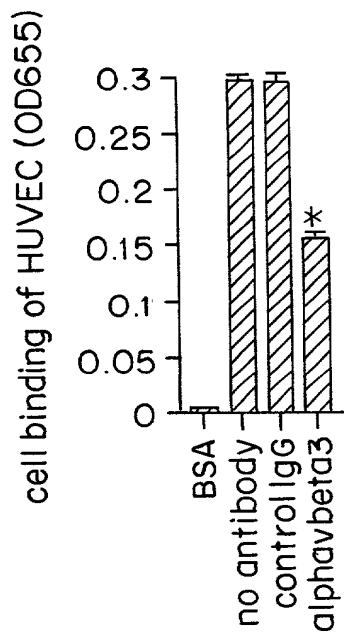


FIG. 44A

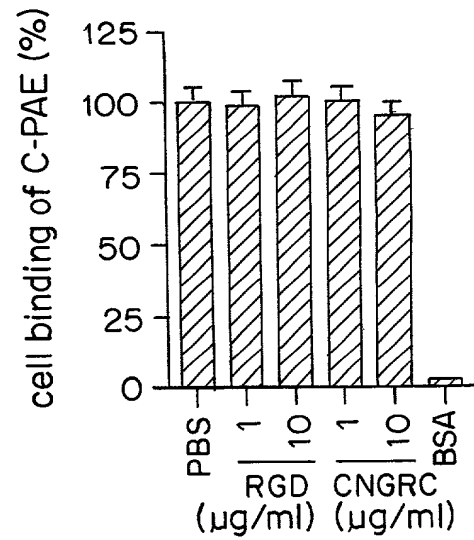


FIG. 44B

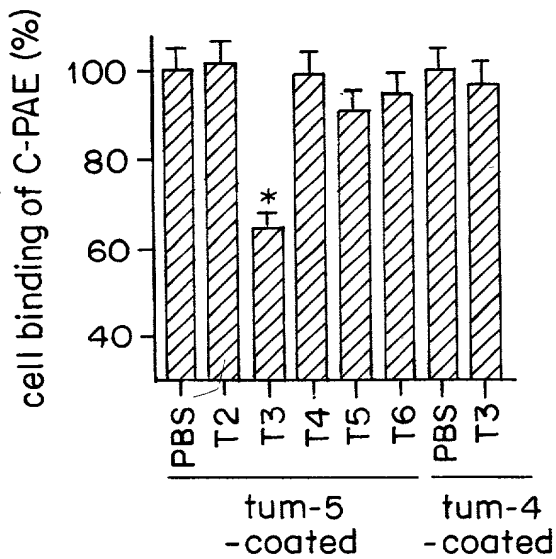


FIG. 44C

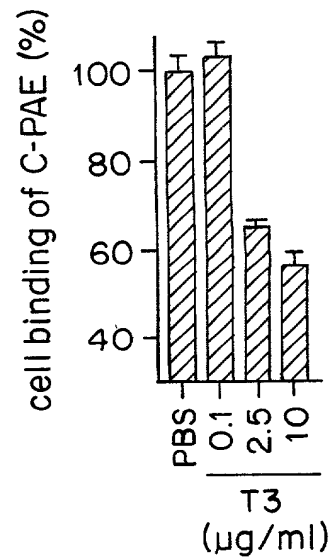


FIG. 44D

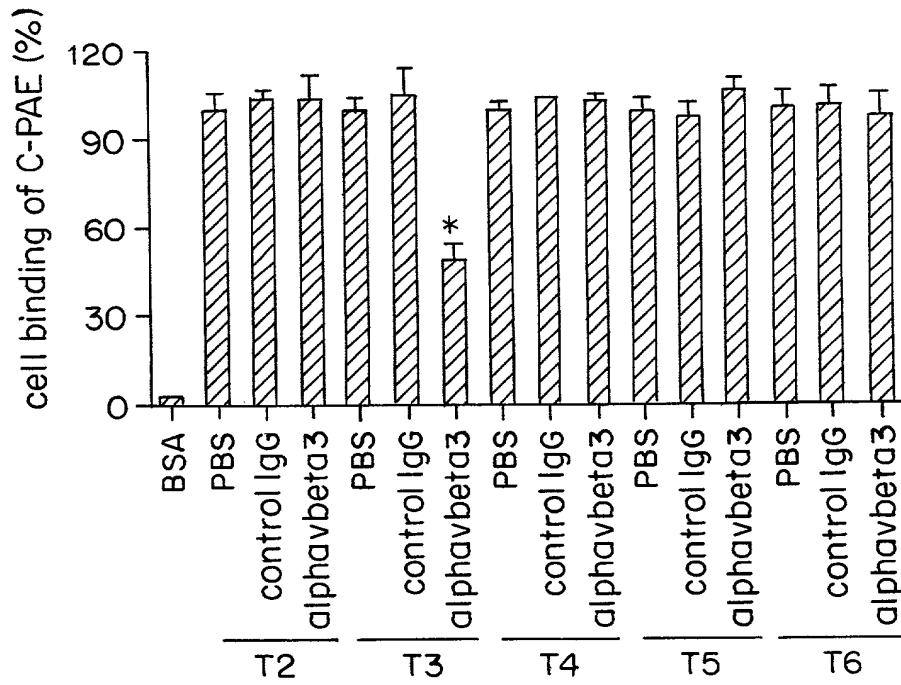


FIG. 44E

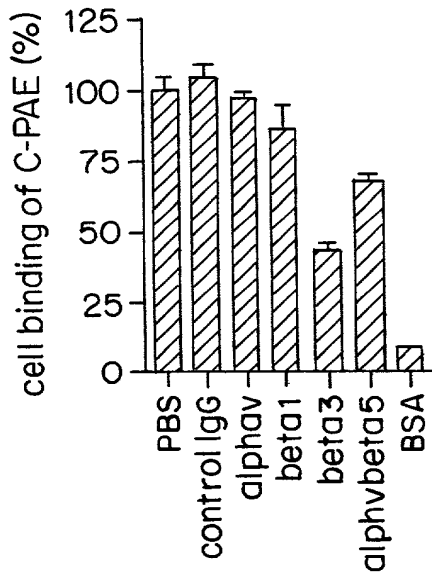


FIG. 44F

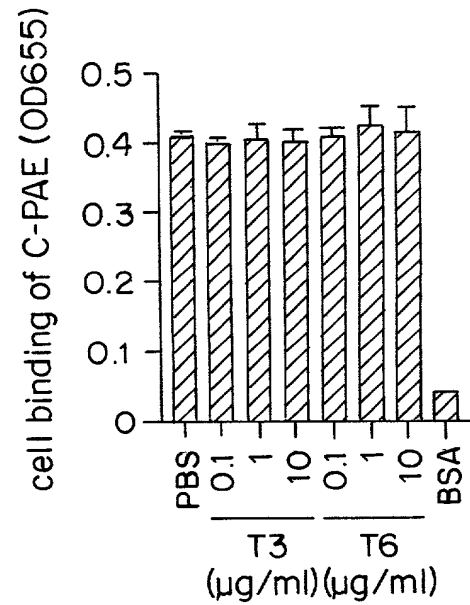


FIG. 44G

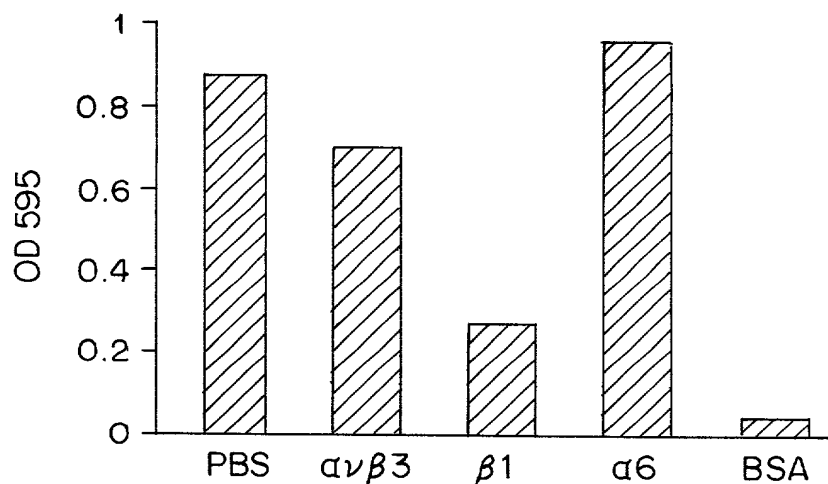


FIG. 45

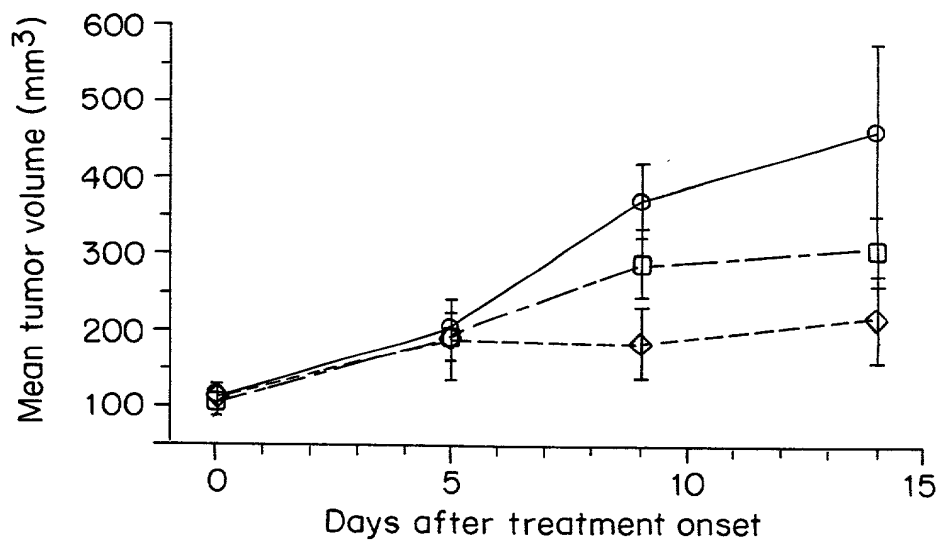


FIG. 46

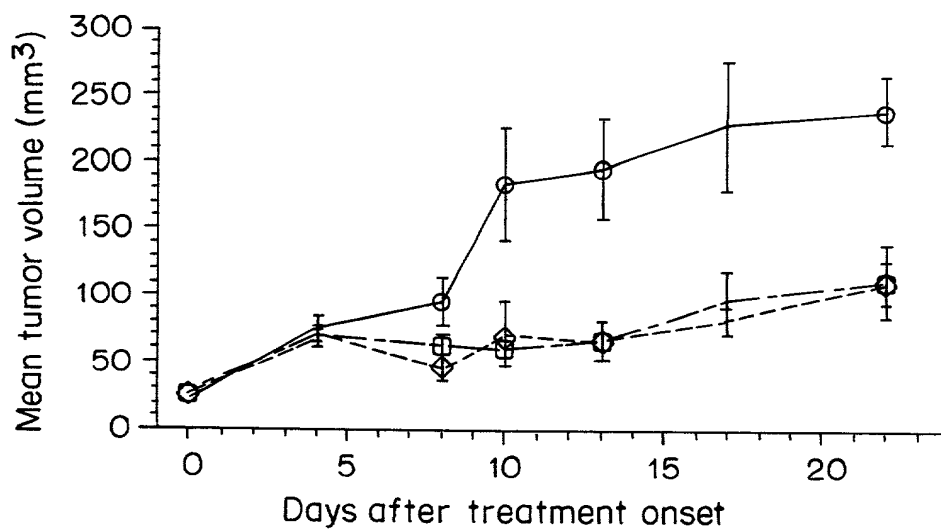


FIG. 47

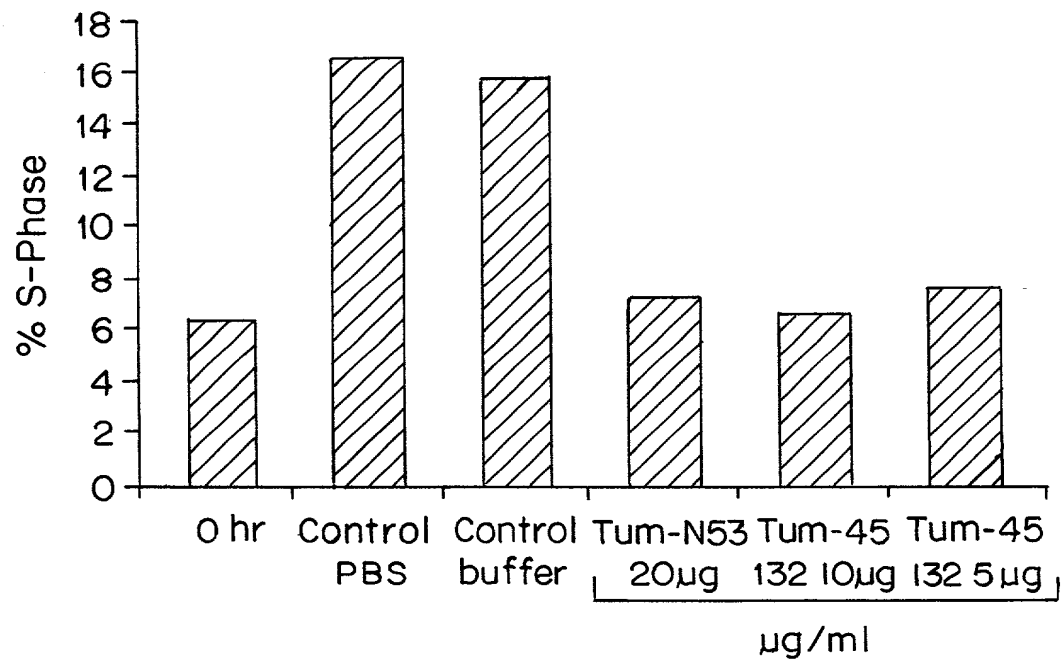


FIG. 48

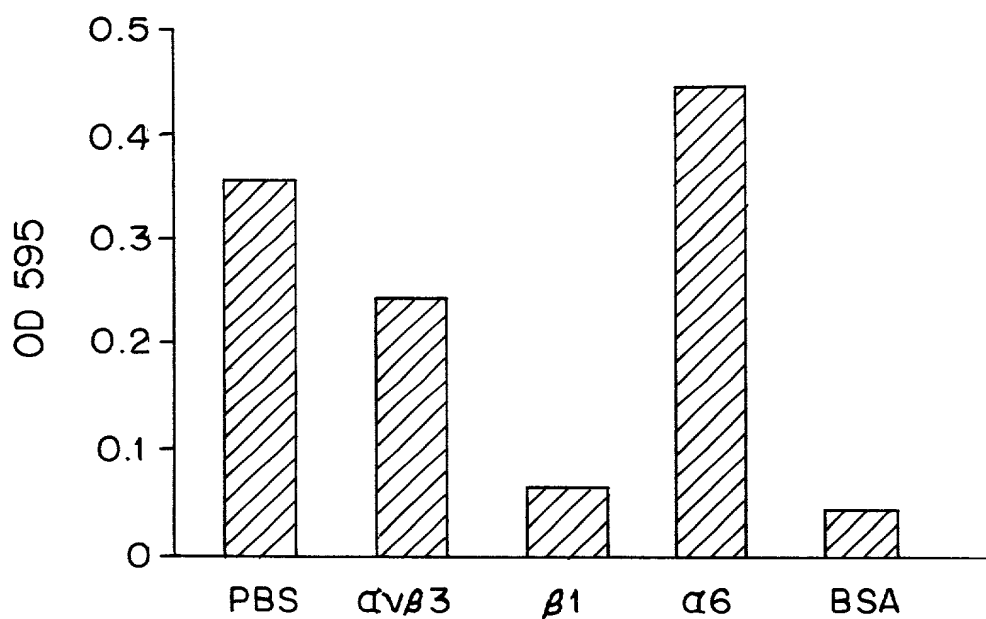


FIG. 49

Fig. 50

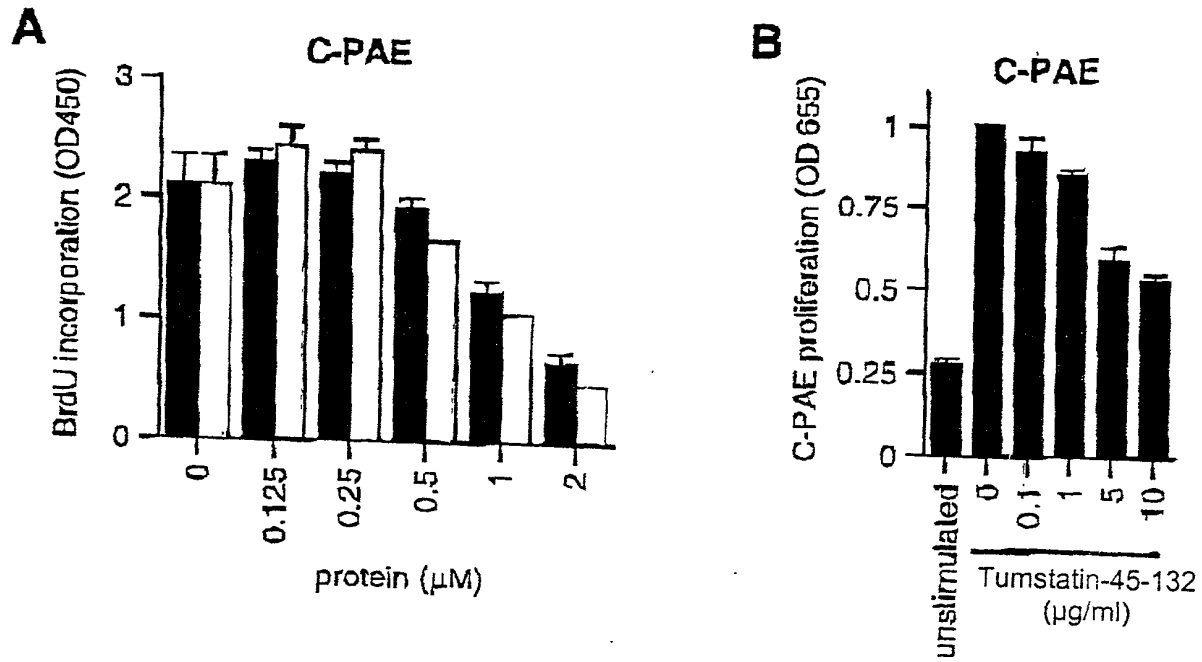


Fig. 51

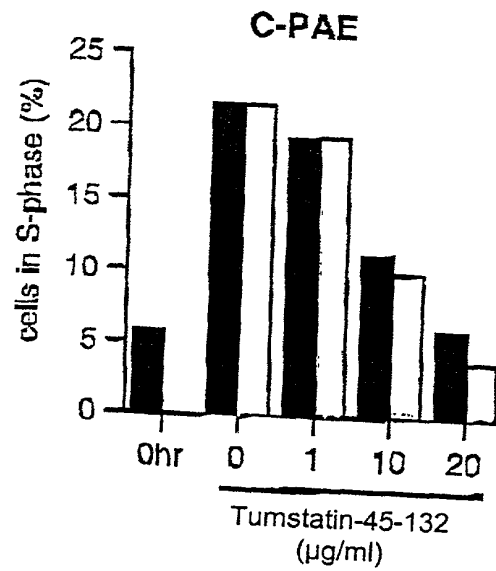


Fig. 52

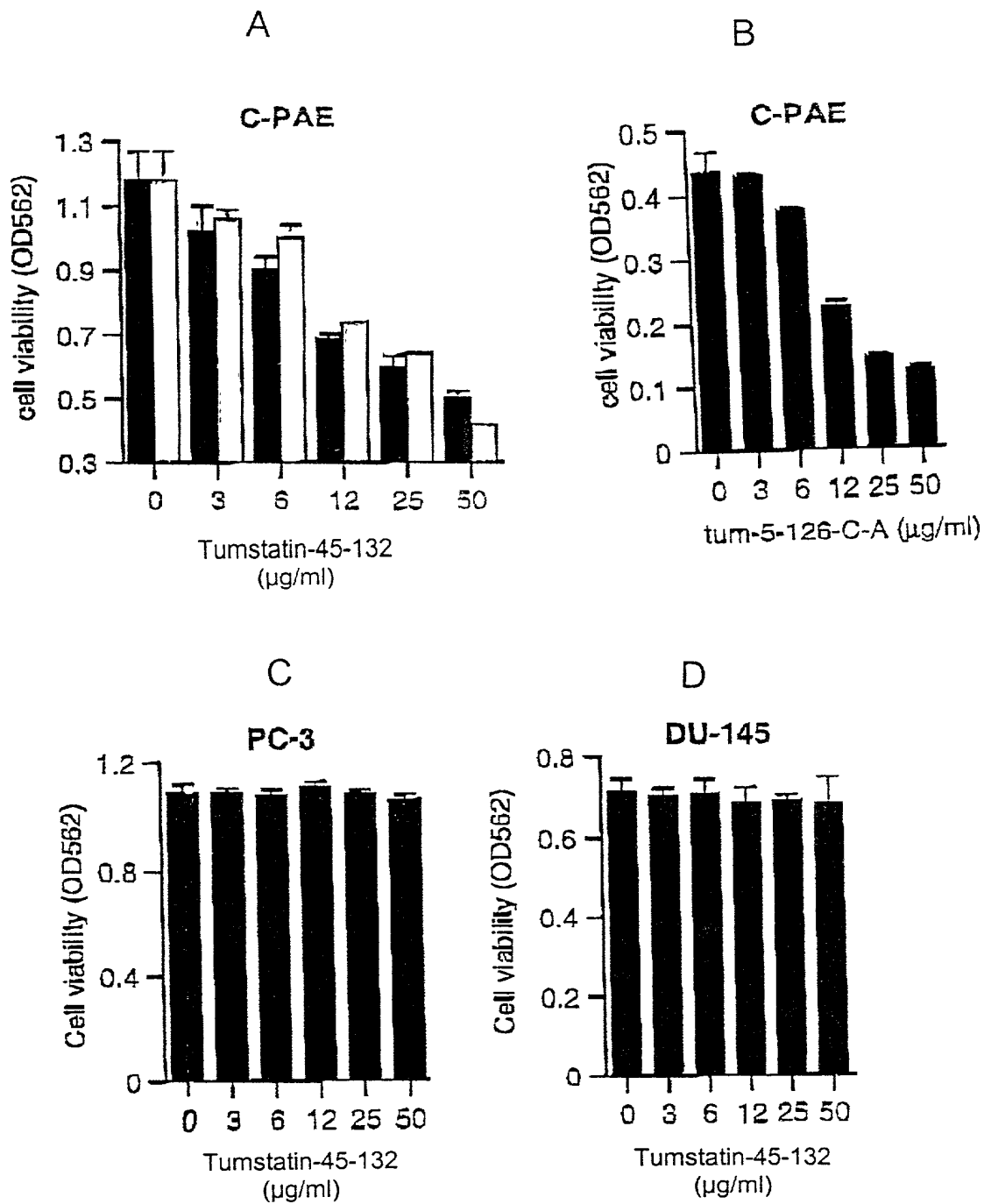


Fig. 54

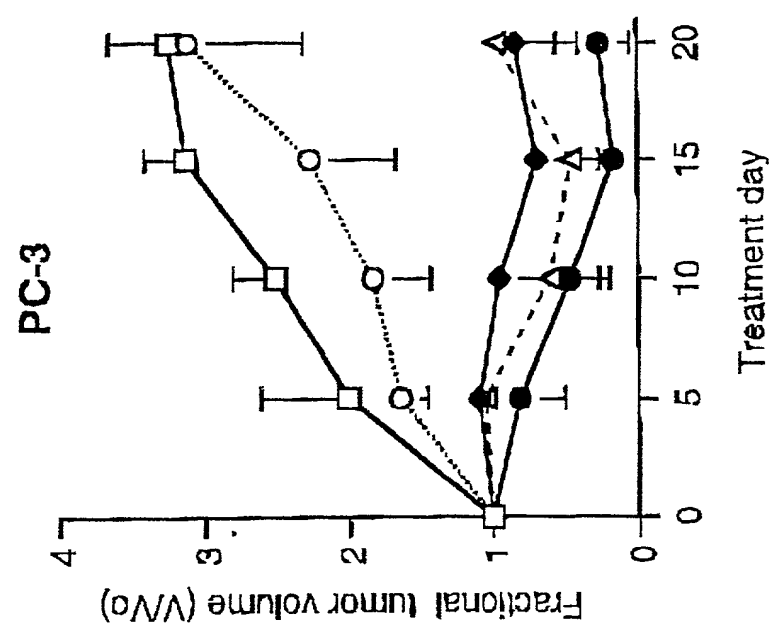
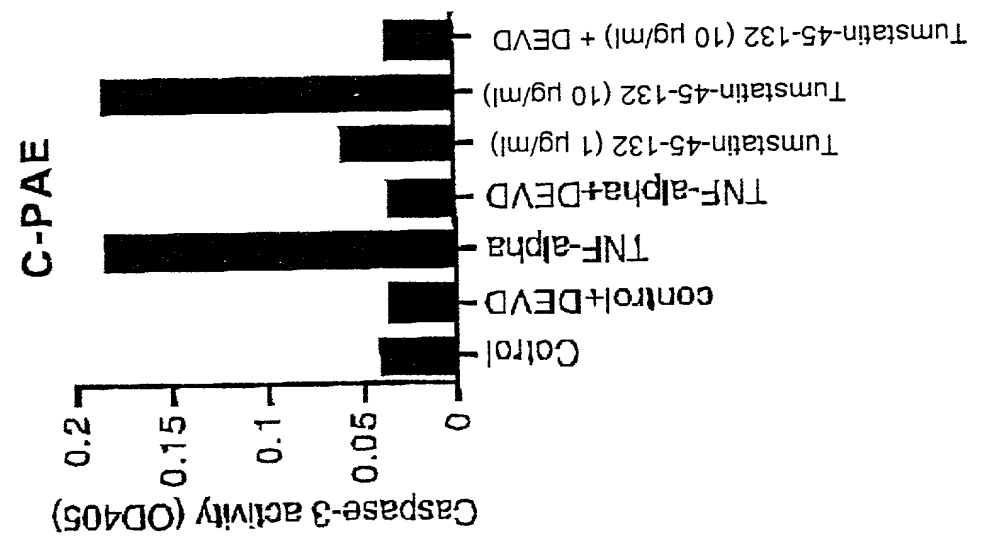


Fig. 53



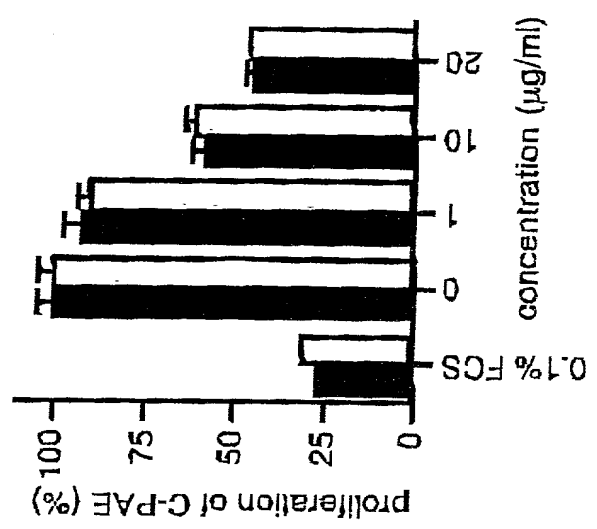


Fig. 56

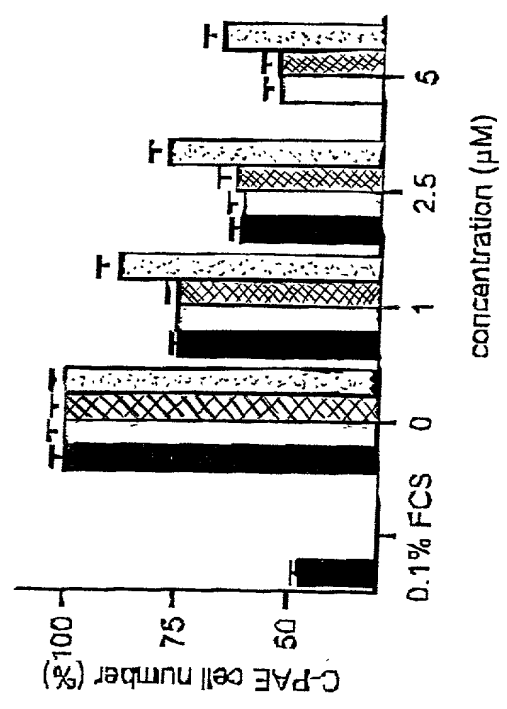


Fig. 57

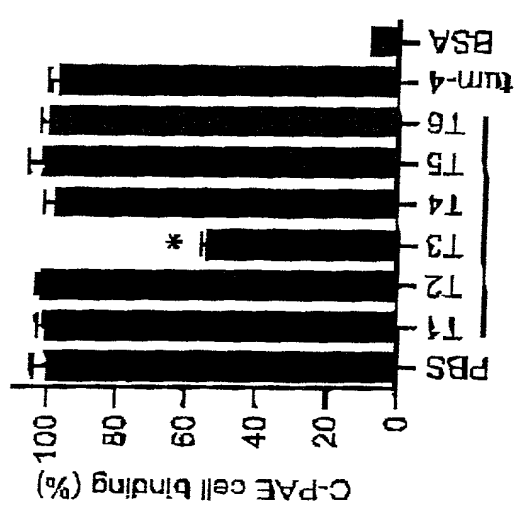


Fig. 55A

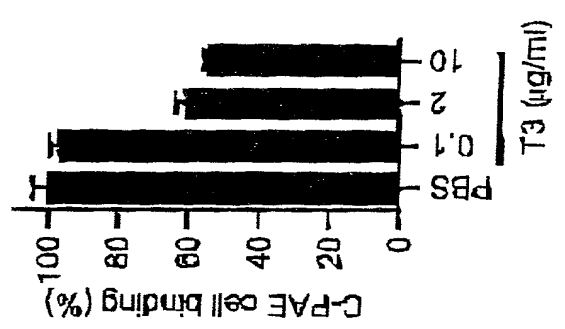


Fig. 55B

Fig. 58A

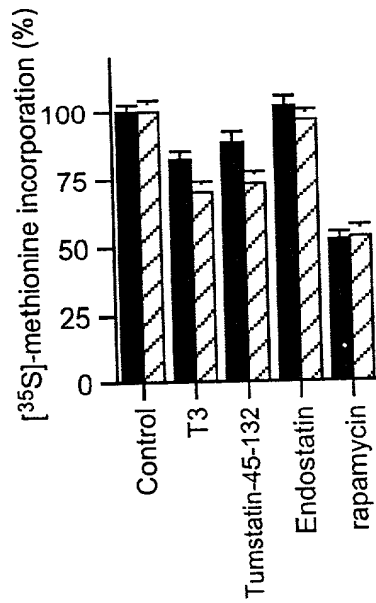


Fig. 58B

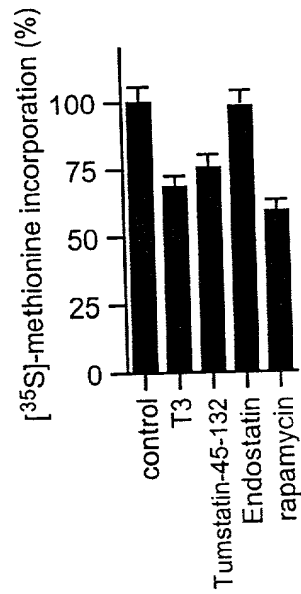


Fig. 58C

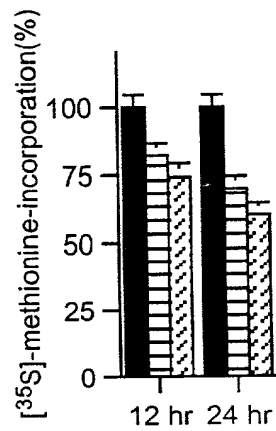


Fig. 58D

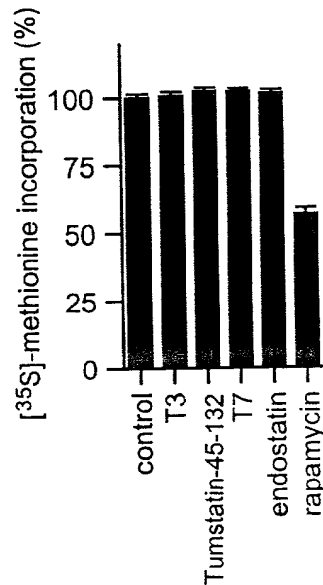


Fig. 58E

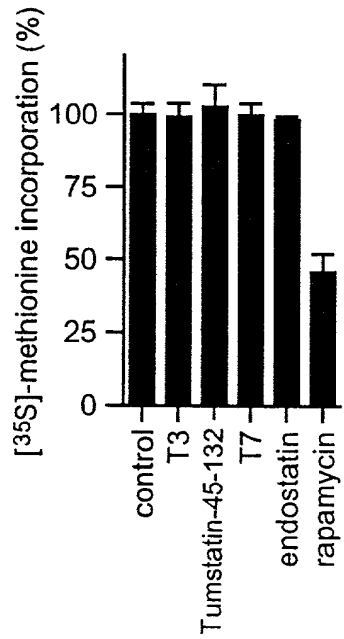


Fig. 58F

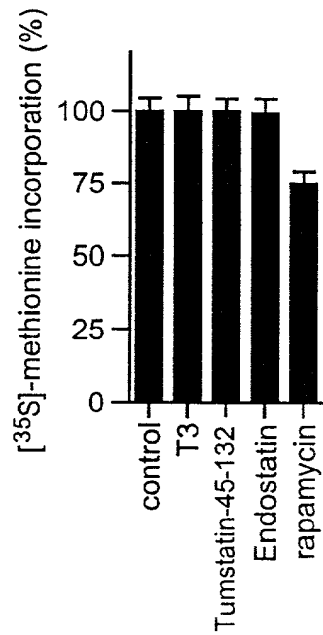


Fig. 58G

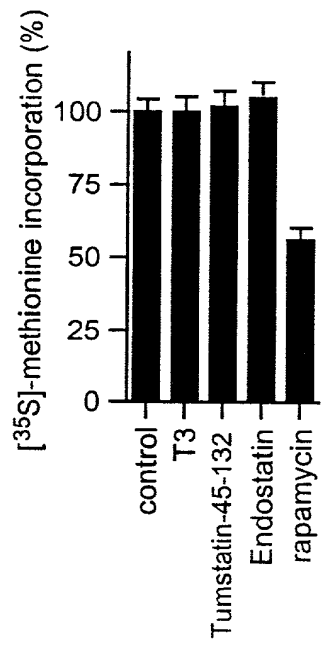


Fig. 58H

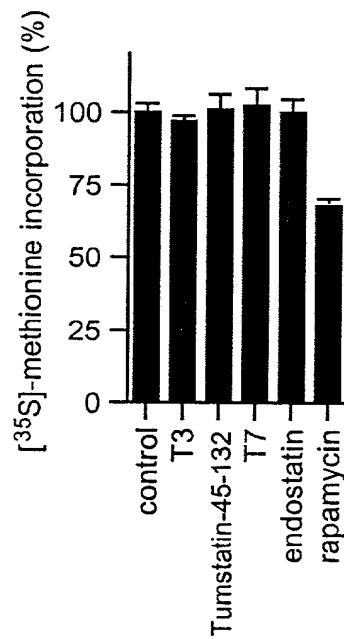


Fig. 59A

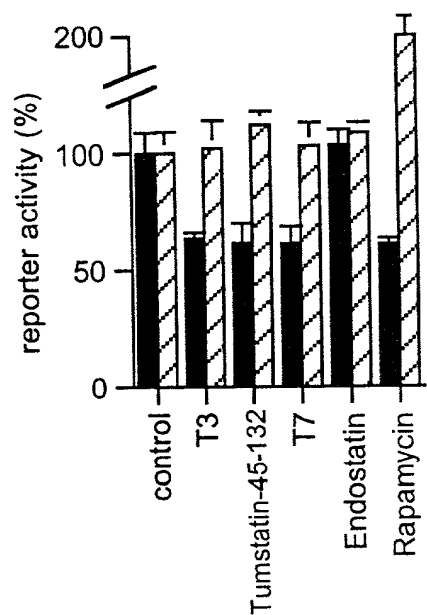


Fig. 59B

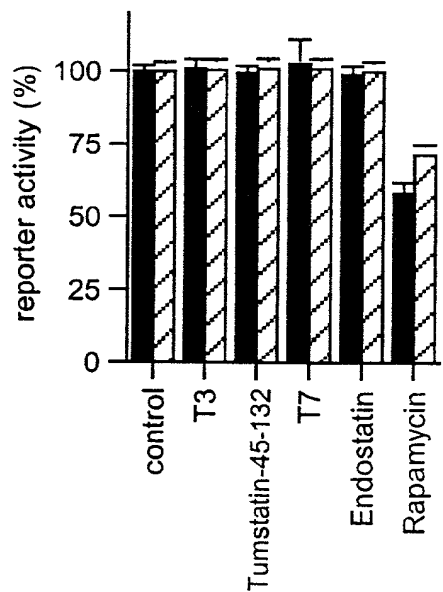


Fig. 60A

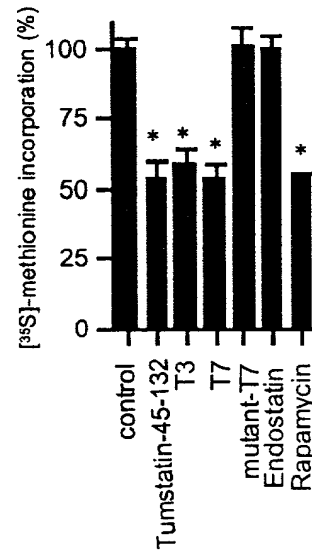


Fig. 60B

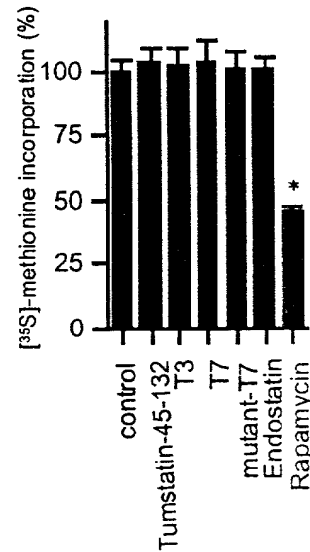


Fig. 60C

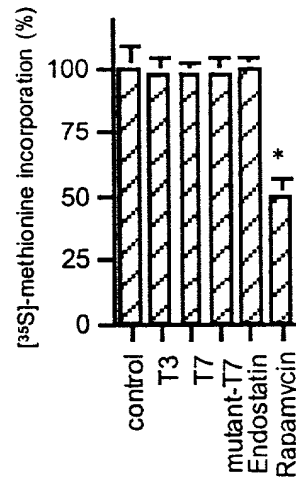


Fig. 60D

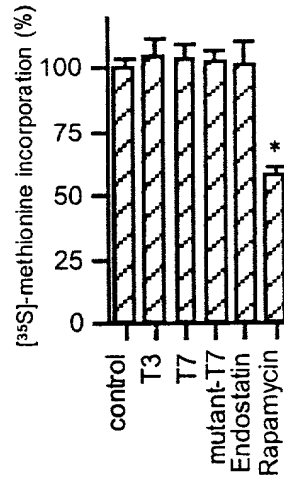


Fig. 60E

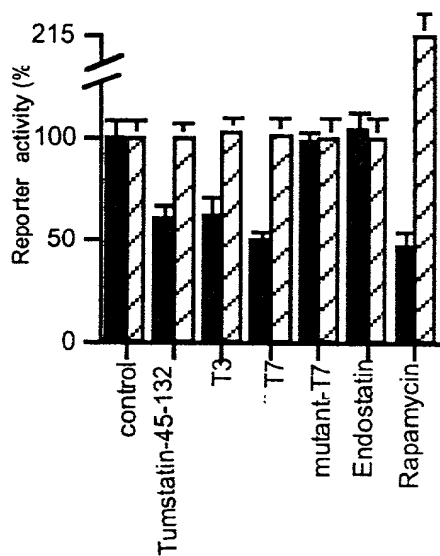


Fig. 60F

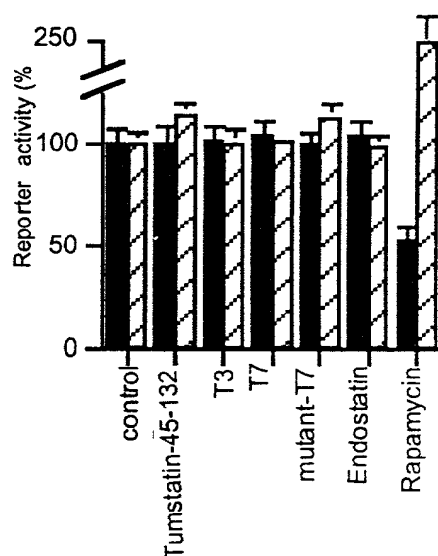


Fig. 60G

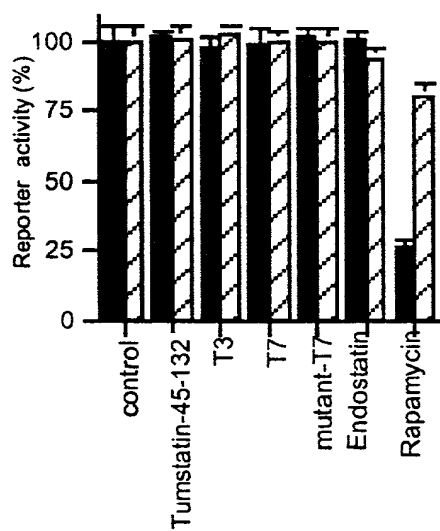


Fig. 60H

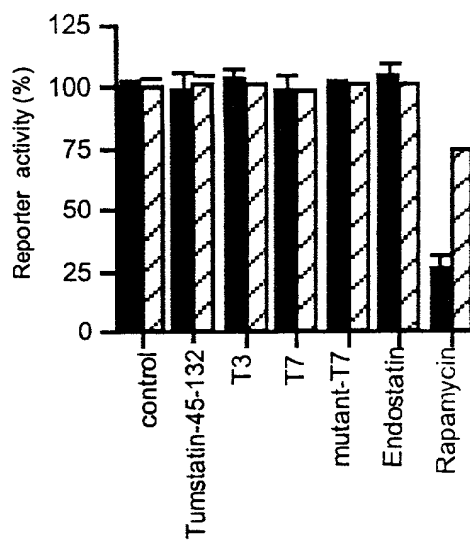


Fig. 61A

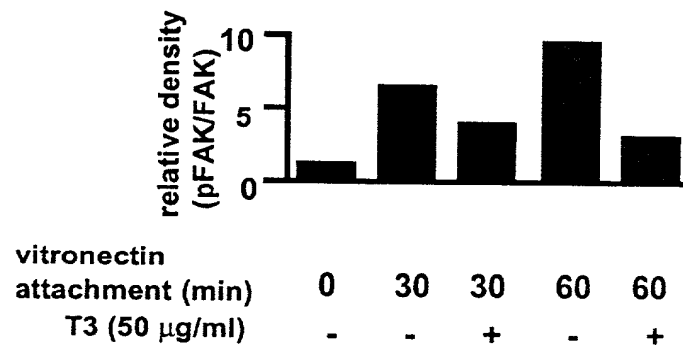


Fig. 61B

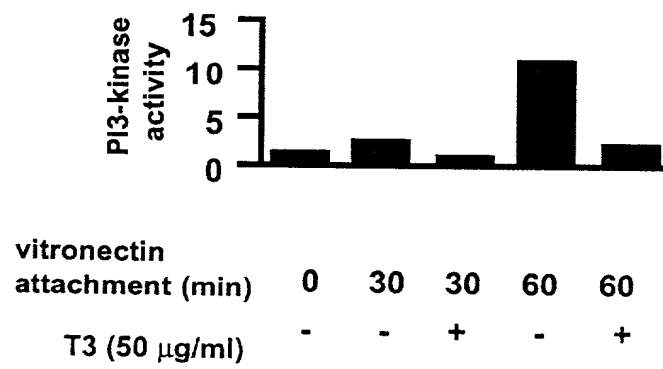


Fig. 61C

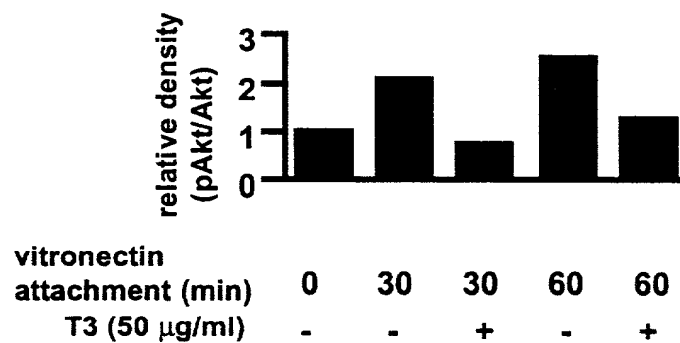


Fig. 61D

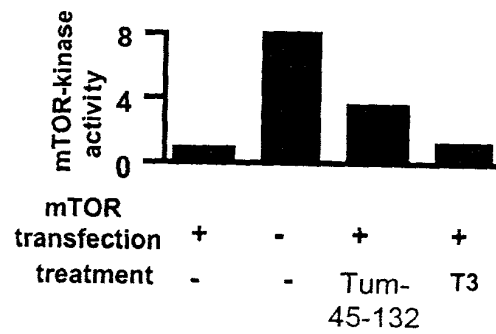


Fig. 61E

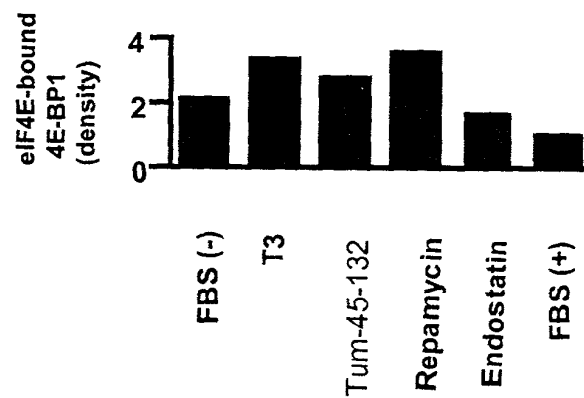
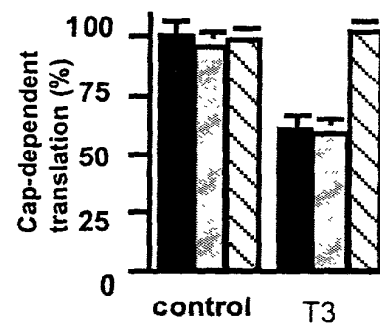


Fig. 61F



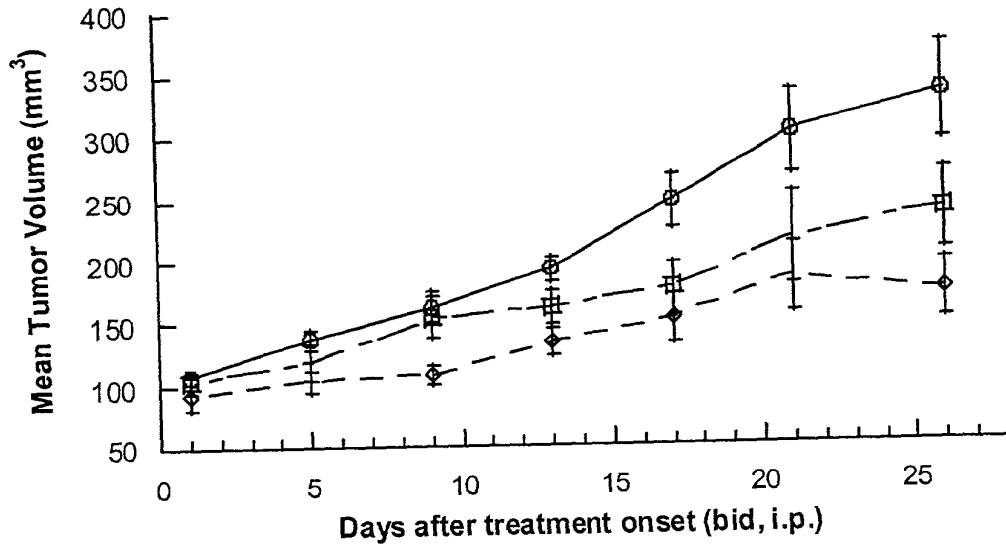


Fig. 62

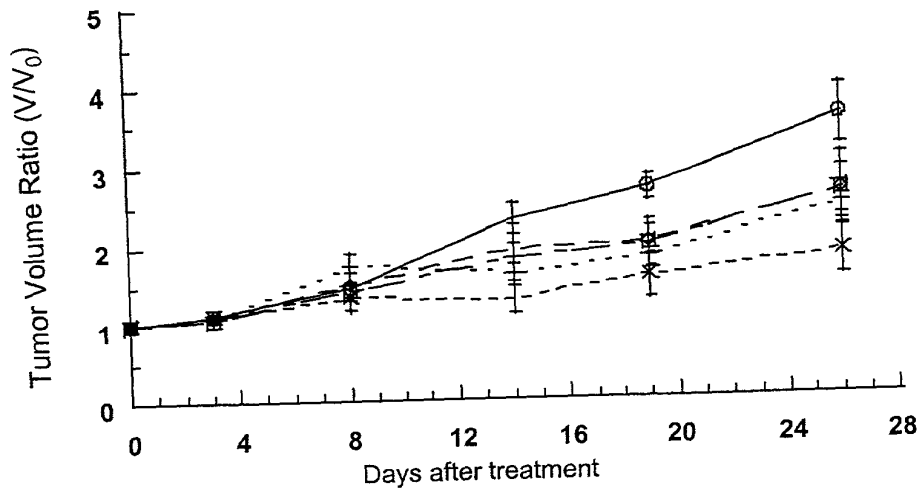


Fig. 63

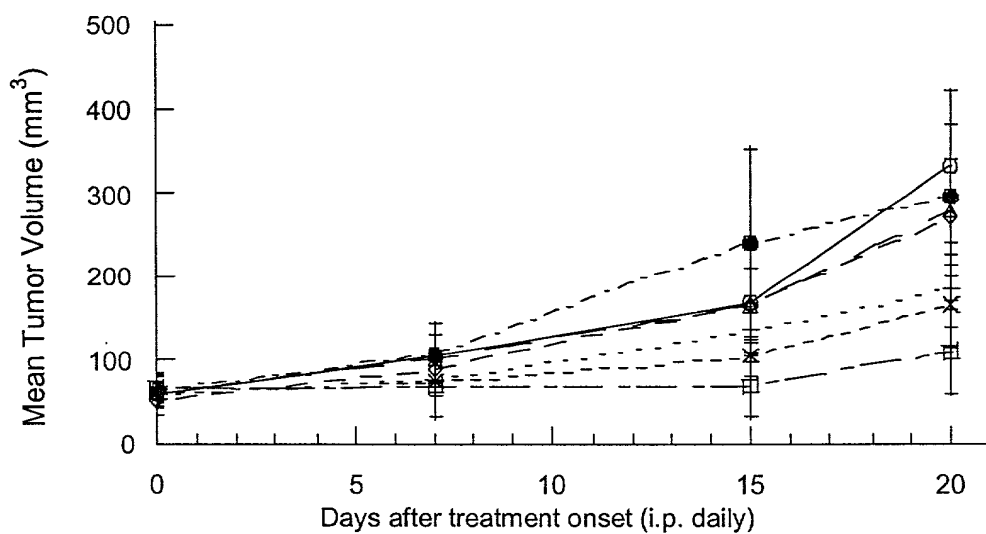


Fig. 64A

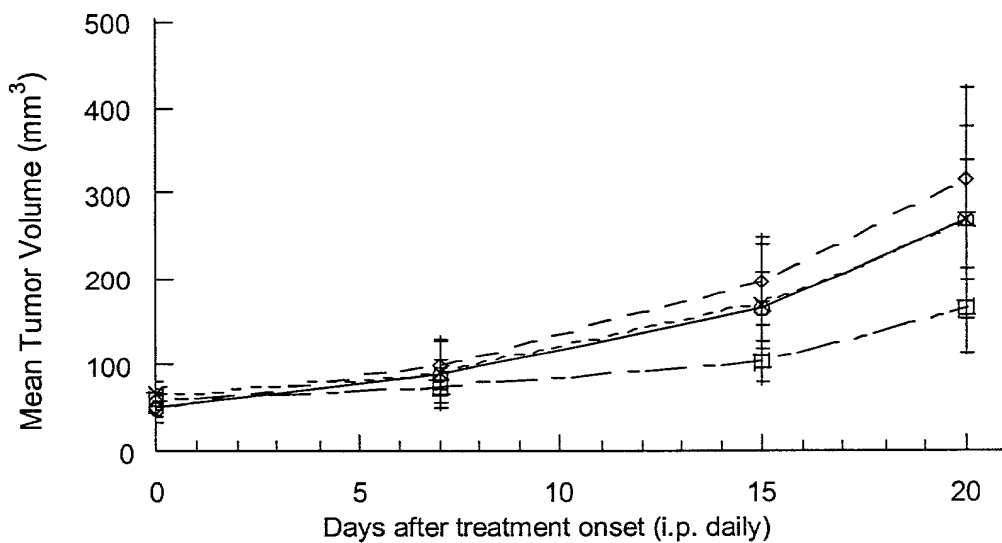


Fig. 64B

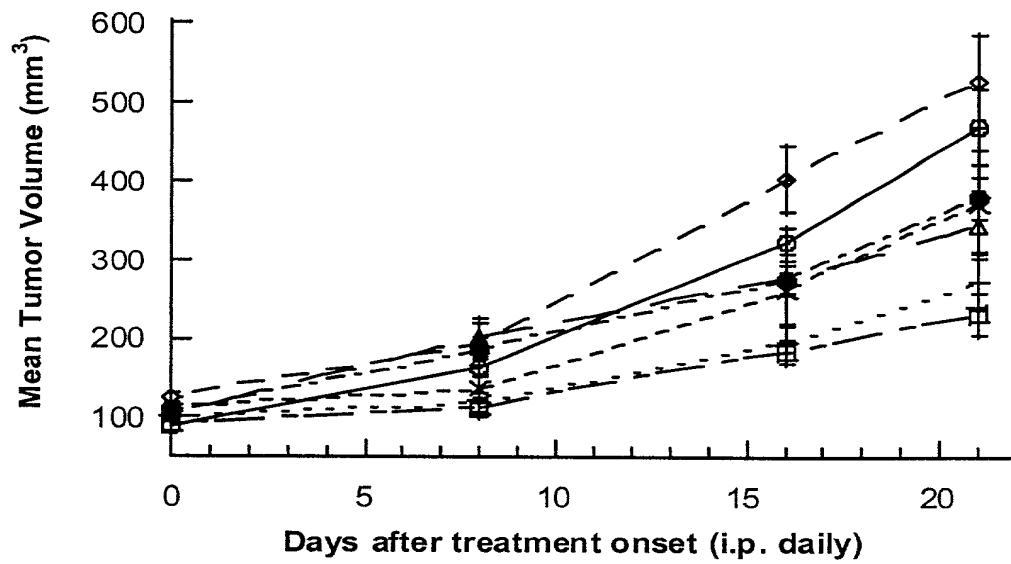


Fig. 65A

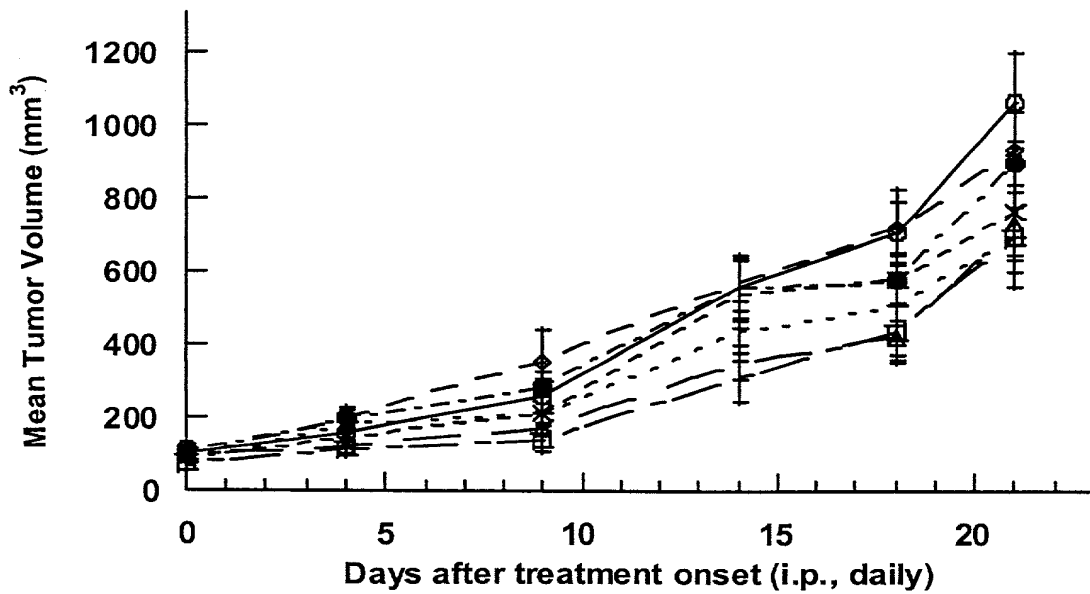


Fig. 65B